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PATHOLOGY

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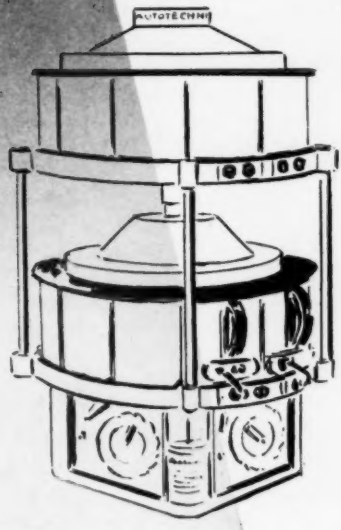

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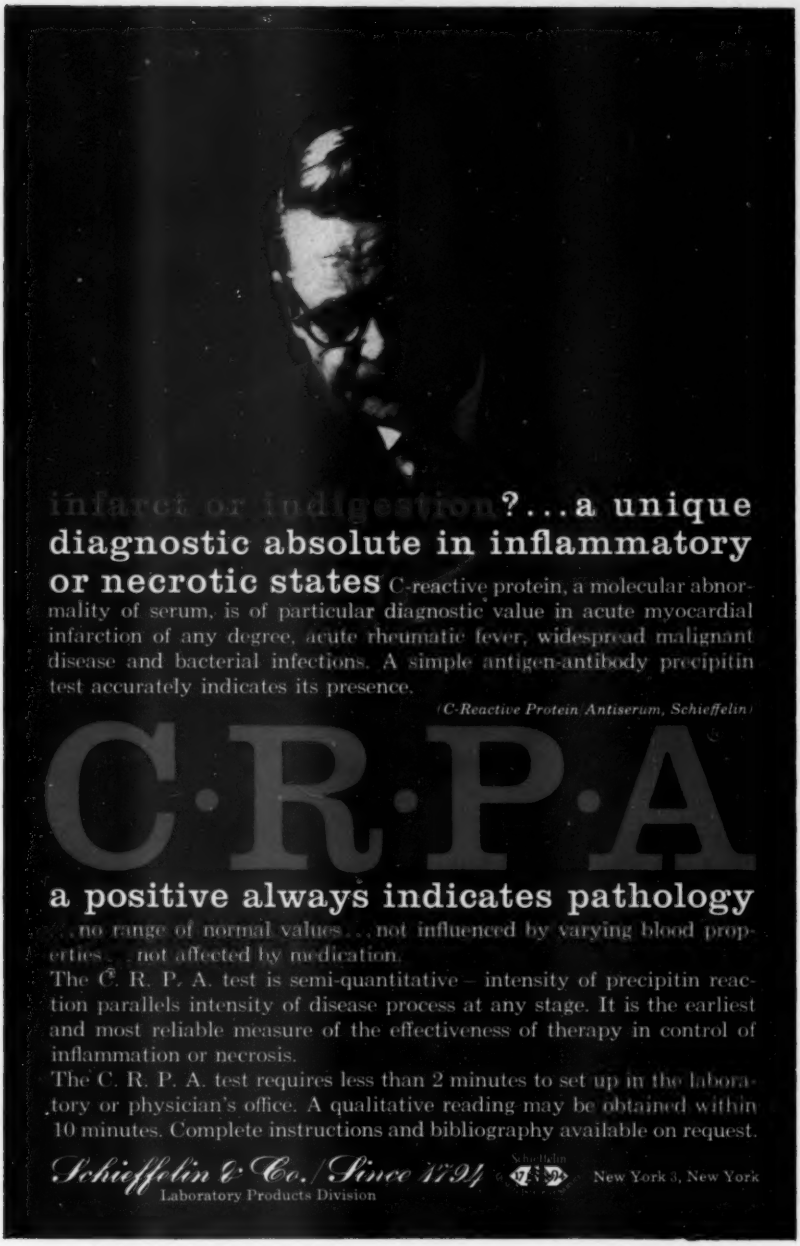

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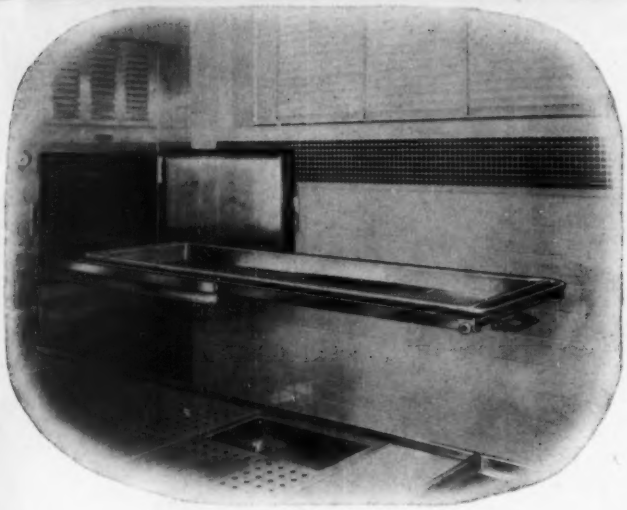

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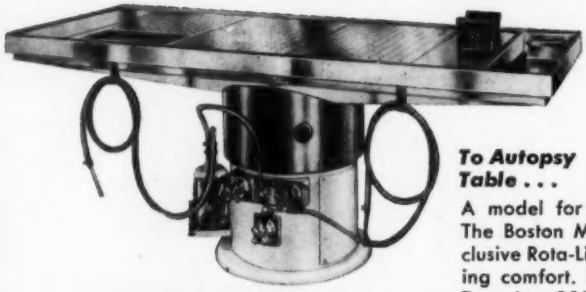

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Unusual Manifestations of Gout

R. C. ROSENQUIST, M.D.; C. S. SMALL, M.D., and P. H. DEEB, M.D, Loma Linda, Calif.

Much has been written on gout, mostly from the clinical standpoint. Few really comprehensive reports have been published in English on the tissue pathology of the disease. Among the best is the report by Lichtenstein, Scott, and Levin.[1]

The purpose of this paper is to report an unusually severe case of chronic gout from the clinical, radiologic, and pathologic points of view. Whereas marked roentgen findings occur in a minority of the cases, this case presents severe and advanced destruction of many of the joints.

The common pathologic features of gout are deposition of urates in joint cartilages, and in periarticular and perichondrial tissues, as tophi. Such tophi, in about 5% of cases, attain large size, and seriously cripple the patient by rendering joint motion painful.[2] Large tophi often ulcerate and drain serous fluid containing urate crystals. Such crystals, when made of fine needles, are only faintly birefringent, but when in broad sheaves and plates, are brilliantly so (Figs. 20 and 21).

Renal lesions are almost constant in chronic gout, and, according to Talbott,[2] are the principal cause of death from the disease. The kidneys are shrunken, scarred, and often laden with urate deposits in the medulla.

Received for publication May 21, 1958.

From the Departments of Medicine, Pathology, and Radiology, respectively, of the College of Medical Evangelists and the Loma Linda Sanitarium and Hospital.

The urate deposits in articular cartilages frequently excite sufficient inflammation to erode subjacent bone, producing sharply defined radiolucent areas in the ends of bones. These deposits excite a tissue reaction, consisting of fibrosis, lymphocyte infiltration, and foreign-body-giant-cell accumulation.

Roentgen Findings

Various authors state that the metatarsophalangeal joint of the great toe is most frequently affected; however, other joints, particularly those of the feet and ankles, and of the hands and wrists, are common sites. As the disease progresses, joints may enlarge. The articular cartilage and the synovial membranes demonstrate deposits of crystals on the surfaces. Hyperplasia is caused by the membrane irritation, and granulation-tissue pannus will grow about the articular cartilage. The margins of the articular cortex may be destroyed, producing rounded lesions, suggesting cysts. The progressive growth of pannus will eventually destroy the cartilage. Golden [4] states that about one-third of advanced cases of gout show roentgenological evidence sufficient to allow a diagnosis to be made. Cyst-like areas may vary in size from a few millimeters to over a centimeter or more in diameter. The production of these cysts, which on the roentgenogram appear as punched-out areas, is caused by the damage to the cortex of the bone by granulation tissue. If the amount of tophus is extensive,

the ends of the bones may be eroded. Bartels[8] demonstrates a case with slight calcification at the olecranon bursa and also a case with tophus and calcification at the base of the proximal phalanx of the foot. Smaller joints apparently are most frequently affected. Some authors have stated that gouty change is not often seen in the larger joints. Radiographic appearances are not pathognomonic but may appear similar to such diseases as rheumatoid arthritis, degenerative arthritis, and Boeck's sarcoid.

Report of a Case

The patient was a 70-year-old Caucasian of German descent, whose occupations have varied from ranching, and managing an Indian trading post, to "safe-cracking." He had had malaria at the age of 32, while in Africa, but his medical history was not otherwise remarkable until the development of recurring attacks of joint pain, first noted when he was about 50 years of age. The attacks of pain were characterized by a sudden onset, unrelated to trauma or any other factor of which he was aware. The toes, knees, and ankles were initially involved in the acute arthritis, the involved joints being acutely swollen, warm, and painful. The individual attacks varied in duration from one day to as long as three weeks. About five years after the initial onset of these attacks, small nodules were noted adjacent to the joints of his hands and feet. Codeine afforded greatest relief for the acute attacks. Corticotropin and cortisone, which were later used, gave no definite relief. Colchicine consistently gave relief.

Progressive enlargement of the tophi occurred, and when the patient was 65 (approximately 15 years after the initial episode of joint pain), painful ulceration and chronic draining sinuses developed over the tophi on his hands and feet. For this reason, the right fourth and fifth toes, and the left fourth toe, were amputated. His debility increased to the point where he was unable to walk, even with crutches.

There was no family history of gout or other hereditary disease. The patient drank a pint of beer a day and had done so for the past 20 years. His dietary history was not remarkable.

Physical Examination.—Height, 6 ft.; weight, 127 lb.; temperature, 96.2 F; pulse, 76 a minute; respiration, 16 a minute; blood pressure, 120/65. The patient was pale and emaciated, with obvious massive tophi of the hands and feet. The skin was dry, with scattered areas of fine scaling, and a faintly yellowish tinge. The sclerae were not icteric. The eye findings were normal except for an arteriovenous ratio of 1:3. No tophi were seen on the ears or nose.

Mouth: The remaining teeth were in good repair; there was a partial lower denture.

Neck and Chest: The cervical nodes were palpable. The thyroid was not enlarged. The lungs were clear to percussion and auscultation. The heart was not enlarged and had a regular sinus rhythm. A Grade 4 harsh, blowing apical systolic murmur was heard.

Abdomen: The liver descended to 3 cm. below the right costal margin and was nontender.

Genitalia: Atrophy of the left testicle.

Rectal: No mass palpable; prostate not enlarged.

Extremities: Posterior tibialis and dorsalis pedis pulses detectable, though faint. The massive tophi involving the feet and hands are illustrated in the accompanying figures. Nodules were detected along the ulnar aspect of the forearm as well.

Laboratory Studies.—The initial blood count showed a hemoglobin of 7 gm. per 100 ml. (47%); hematocrit 21 vol. %. Leukocytes 6,450 per cubic millimeter, with 18% lymphocytes, 5% monocytes and 1% eosinophils, and 76% neutrophils. Specific gravity of urine 1.014; pH 4.8; protein 2%. The microscopic field was packed with pus cells and blood. The initial blood nonprotein nitrogen was 141 mg. per 100 ml. Serum uric acid, 9.1 mg. per 100 ml.; CO_2-combining power, 33 vol. % (15 mEq/L.); potassium 19.1 mg. per 100 ml. (4.9 mEq/L.); calcium 11 mg. per 100 ml. (5.5 mEq/L.); inorganic phosphorus 4.5 mg. per 100 ml. Wassermann and Kahn reactions, negative.

Course of Illness.—The patient was started on probenecid, 0.5 gm. daily, with supplemental sodium bicarbonate to keep the urinary pH neutral or alkaline. The program was not closely followed because of gastrointestinal irritation. The patient was seen on five subsequent hospitalizations, during which time his condition progressively deteriorated. The pain in his extremities and low back became almost continuous. Serum uric acid levels were consistently elevated, being in the range of 8 to 10 mg. per 100 ml. Fasting blood sugar was 123 mg. per 100 ml. The urine consistently showed protein, blood, and pus. At the time of his final hospitalization, the nonprotein nitrogen was 114 mg. per 100 ml. His chemical findings were otherwise unchanged. He died Sept. 2, 1956.

Radiographic Findings

The radiographic studies included multiple views of the hands, elbows, knees, and feet and showed remarkably extensive calcium deposits, as well as areas of bone destruction and severe distortion of joint relationships. The views of the hands showed the widespread distribution of the calcific deposits and the areas of bone destruction with subluxation. Particularly, this involved the thumbs of both

Figure 1

Fig. 1.—Both hands. Distortion of the articular aspects, particularly at the metacarpophalangeal joint area, and the subluxation elsewhere, are seen. Note the deposits near the distal phalanges of the index and middle fingers.

Figure 2

Fig. 2.—Left hand showing more detail. Even larger deposits are present, with severe destruction and distortion at the thumb. The left carpal region shows less involvement than the right.

hands and some of the metacarpophalangeal joints. The rather advanced arteriosclerotic changes in the vessels were also noted (Fig. 1). The left hand showed extensive deposits involving the index and fifth fingers, with the severe destruction at the metacarpophalangeal joint of the thumb (Fig. 2). The soft-tissue swelling and the calcification in the bursal sacs were demonstrated in the elbow region bilaterally, and along the shafts of the bones further abnormal densities were seen (Figs. 3 and 4). In the elbow region the joint relationships were fairly well preserved, however. The right knee showed greater amounts of calcium deposit than the left, but along the femoral shaft and, to a less extent, about the joint space there was, again, evidence of the wide distribution of the tophi. The left knee joint was preserved, although the space was narrowed. The right knee joint showed greater changes involving the articular surfaces (Figs. 5 and 6). Both feet (Fig. 7) showed deposits from the region about the distal ends of the tibia and fibula to the interphalangeal joints. The destructive processes, as well as the extensive calcium deposits, were most evident at the ends of the metatarsal bones. However, the relationships of the phalanges showed a bizarre character. The calcium-laden tophi seemed to be scattered indiscriminately about the tarsal area and in the soft

Fig. 3.—Right elbow. Note extensive calcification of the bursal sac and additional tophi on the volar aspect of the joint.

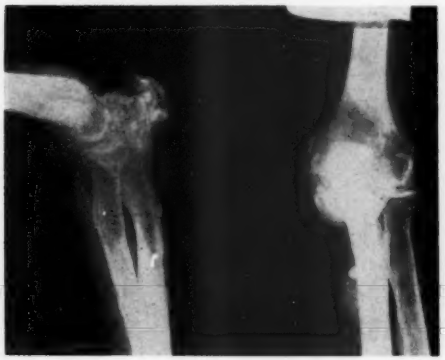


Fig. 4.—Left elbow. Less dense, but extensive, tophi are demonstrated. Note also soft-tissue swelling. The joint surfaces of the elbow are fairly well preserved.

Figure 5

Fig. 5.—Both knees. Views of both knees show the surfaces paved with urate deposit, as seen on the gross specimen (Fig. 14). Extensive deposits are seen along the femoral shaft on the right. Some narrowing of the joint space is suggested.

Fig. 6.—Right knee. The right knee shows the massive, dense shadows above the patella, and additional densities near the articular surfaces of the femur and tibia. Also noted is the calcification of the popliteal vessels.

Figure 6

tissues surrounding the foot. The lateral view of the right foot (Fig. 8) demonstrated the large amount of material on the dosal aspect at the distal ends of the metatarsal bones and scattered more uniformly at the tarsal area.

Autopsy Findings

Autopsy was performed 27 hours after death and is reported as follows: Height, 6 ft.; weight, about 110 lb. Skin is smooth, but dry and sallow. Subcutaneous fat is very scanty. Numerous hard lumps, up to 5 cm., are seen under the skin of the hands near joints (Fig. 9). Ulcers draining thin pus surmount several of these lumps. When cut, the lumps are seen to be white and faintly gritty, with heavy fibrous septa subdividing them. Crystalline material is easily dug out of these tophi. Some are rather generally calcified. The feet are much

Figure 7

Fig. 7.—Both feet. The anteroposterior views of the feet show extensive deposits, extending from the ankle joint to the metarsophalangeal joint regions. Loss of bone substance and damage to the surfaces are noted.

Fig. 8.—Right foot. Lateral view of the right foot shows the deposits even more clearly, particularly on the dorsal aspects of the tarsal structures, but distributed to some extent throughout the area.

Figure 8

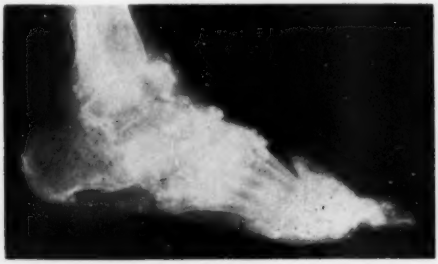


Figure 9

Fig. 9.—Hands, showing numerous tophi with ulcers.

Fig. 10.—Feet, showing tophi, skin ulcers, and white urate coating of ankle joint surface.

Figure 10

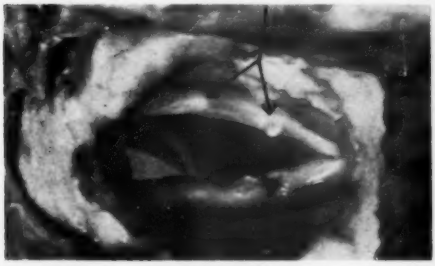


Figure 11

Fig. 11.—Larynx, showing cartilaginous nodules on vocal cords.

Fig. 12.—Shoulder joints, showing urate coating.

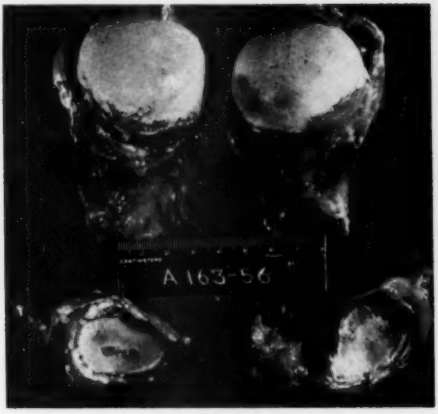
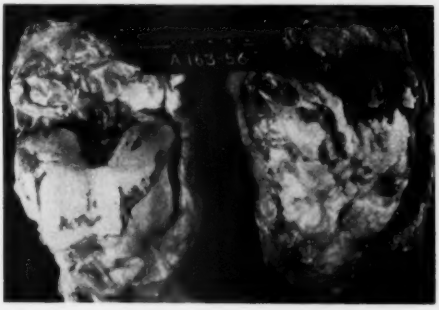


Figure 12

Fig. 13.—Elbow joints, showing urate coating.

Fig. 14.—Knee joints, showing urate coating.



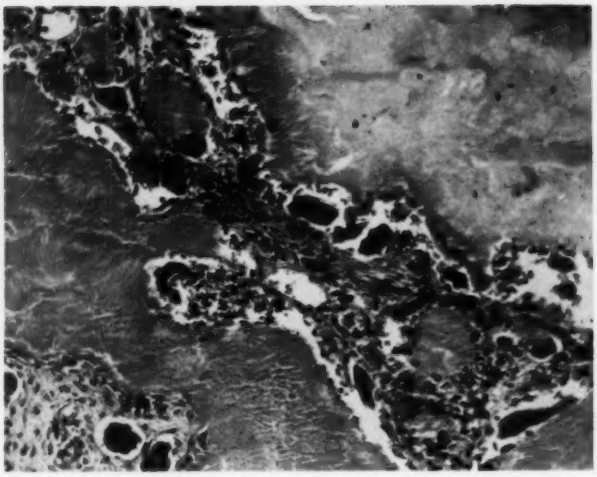


Fig. 15.—Tophus with giant cells. × 130.

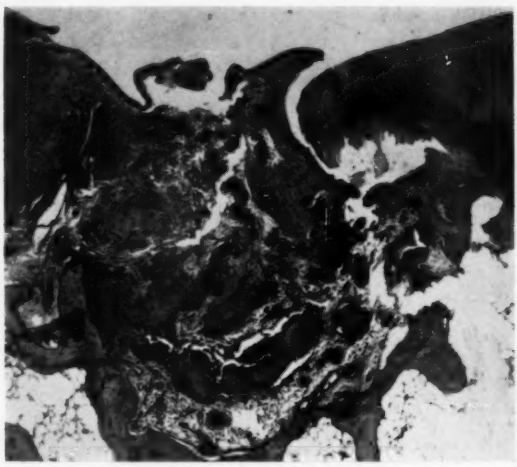


Fig. 16.—Tophus with erosion of joint surface. × 32.

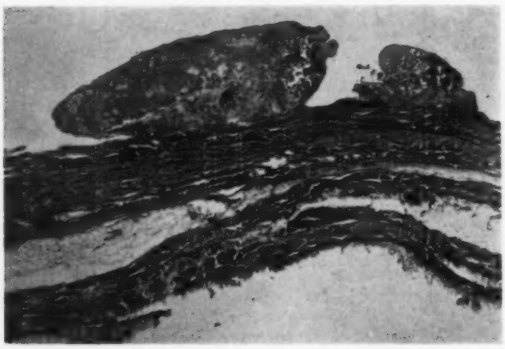


Fig. 17.—Inner aspect of joint capsule with protruding urate nodules. × 10.

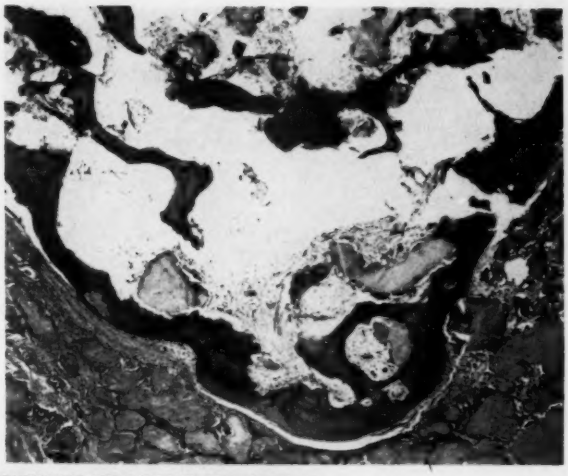


Fig. 18.—Ossification in a subcutaneous tophus. × 25.

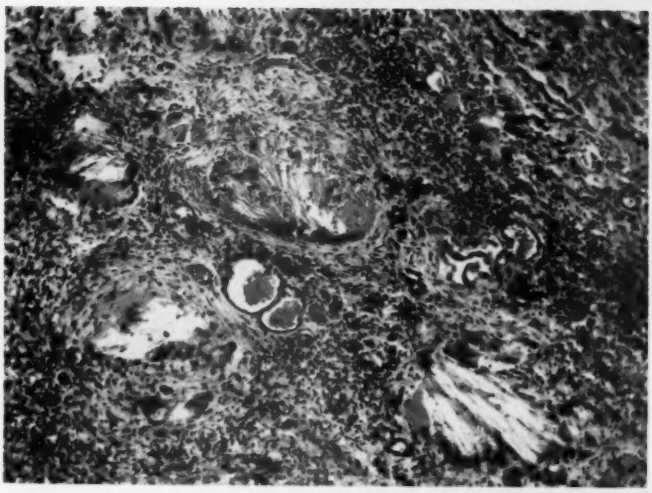


Fig. 19.—Urate crystals in kidney. Reduced to 90% of mag. × 80.

Fig. 20.—Urate crystals in a tophus (unpolarized light). Reduced to 88% of mag. × 30.

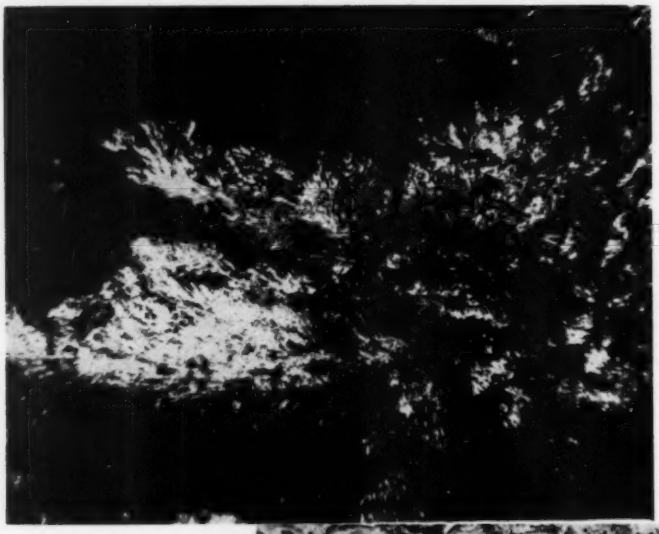


Fig. 21.—Same view as Figure 20 (polarized light). Reduced to 88% of mag. × 30.

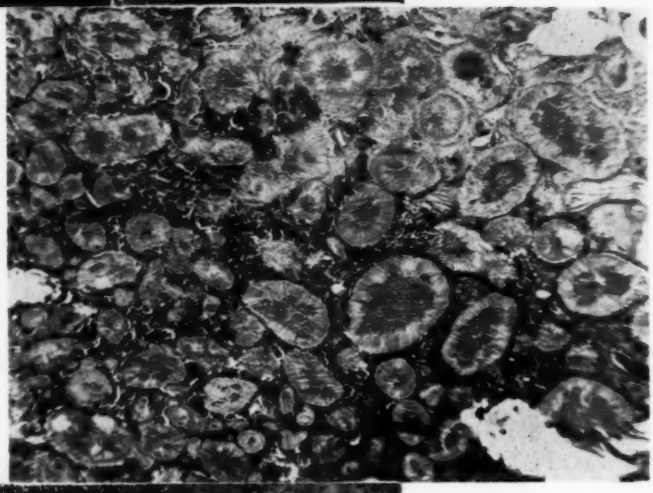


Fig. 22. — Urate crystals in a tophus. Reduced to 88% of mag. × 60.

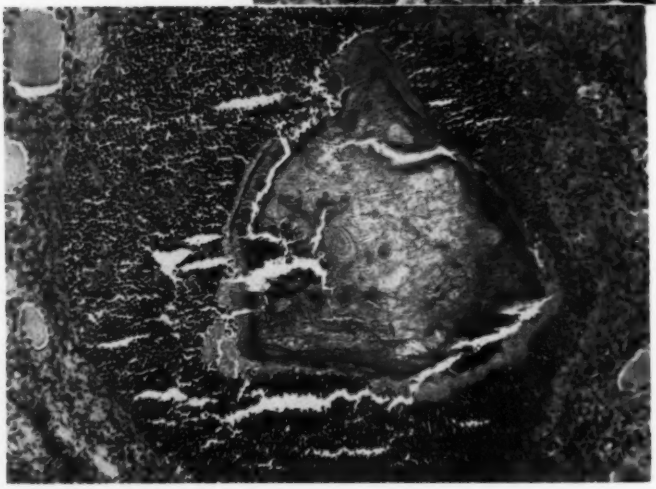


Fig. 23.—Small abscess in kidney containing large urate crystal. Reduced to 90% of mag. × 80.

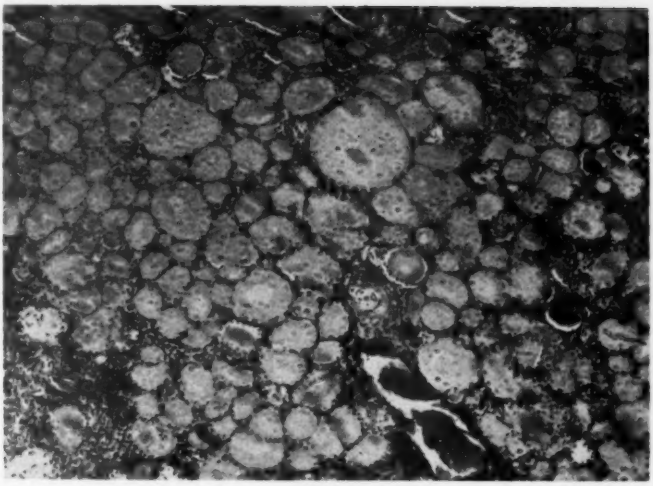


Fig. 24.—Hyaline casts in kidney. Reduced to 88% of mag. × 50.

distorted by similar large tophi on the dorsum and toes, but not on the soles (Fig. 10). Large tophi are found on the elbows, knees, and shoulders. No tophi are seen on the nose or ears.

The brain weighs 1,250 gm. Its arteries are rather thin and smooth and patent. The meninges are smooth and thin. Multiple sections show no gross brain lesion. The spinal cord appears normal.

The pericardium is smooth. The heart weighs 380 gm., with soft brown muscle, containing no infarcts. The left ventricle is 14 mm. thick; the right, 4 mm.; the septum, 12 mm. The coronary orifices are patent, but the left descending artery is 40% narrowed, and the right 60%, by calcified atheromas. The aortic valve is 7 cm. in circumference, and its cusps are calcified at their bases, with fusion of the posterior and left lateral cusps. The ascending aorta is smooth, but the descending aorta shows marked atherosclerosis.

The larynx shows two white nodules, measuring 2 mm., on one vocal cord (Fig. 11). (To our disappointment, these were heterotopic cartilage, and not tophi.) The lungs weigh 400 and 520 gm. and show no lesion.

The digestive tract presents no lesions. The liver weighs 1,200 gm. and shows no lesion.

The spleen weighs 200 gm. and is purple and soft. In both axillae and on the lateral thoracic wall are many firm gray lymph nodes, measuring up to 3 cm., microscopically showing only chronic lymphadenitis, presumably due to the infection of tophi on the hands.

The left kidney weighs 80 gm., the right 100 gm. The capsules strip with slight difficulty, revealing a granular surface, containing a few small cysts. The cortex is 2-3 mm. thick, and the cut-surface markings are quite obscure. In cortex and medulla of both kidneys are a few white streaks, suggesting urate deposits, and many cysts, up to 6 mm., full of gray viscid pus. The ureters are normal. The bladder is quite inflamed and contains 30 ml. of almost pure pus.

The red marrow appears normal in quantity. The thoracic vertebrae have prominent calcific "lipping," which actually results in fusion of the upper six of them. The joint surfaces of the shoulders (Fig. 12), elbows (Fig. 13), wrists, knees (Fig. 14), ankles, and sternoclavicular joints are a uniform pure white with urates, but are very well lubricated with mucin. The juxta-articular tissues contain tophi up to 5 cm. in diameter, especially in the patellar tendons. Near the knees and elbows are several large bursae full of gray fluid and crumbled calcific and chalky material. Microscopic examination reveals many cholesterol crystals in this fluid, but the chemical test for urates was strongly positive.

The thyroid, parathyroids, adrenals, and pituitary show no lesion.

The genital system shows no lesion except some microscopic infarcts in the prostate gland and fibrous atrophy of one testis.

Microscopic study of the tophi reveals large masses of urate needles and crystals surrounded by fibrous tissue, containing many foreign-body giant cells (Fig. 15). The joint surfaces show a uniform coating of urates and many defects in the bone surface (Fig. 16). These defects are produced by urate masses. Other areas show protruding tophaceous nodules on joint surfaces

(Fig. 17). Several tophi are partially calcified, and some are even ossified (Fig. 18).

In some areas great masses of burr-like crystals are seen (Fig. 19). Some crystals are beautifully birefringent (Figs. 20 and 21).

Sections of kidney show scarring, heavy fibrous thickening of artery walls, tophaceous areas in the kidney substance (Fig. 22), and many abscesses containing urate-crystal masses (Fig. 23). Many tubules are plugged with hyalin casts (Fig. 24). The glomeruli are remarkably well preserved, no scars, adhesions, crescents, or significant hyalinization being seen.

Anatomic Diagnosis.—Widespread tophaceous gout; gouty nephritis; uremia; calcific aortic-valve stenosis.

Comment

The remarkable feature of this case is the extremely wide distribution of urate deposits, including coating of all joint surfaces with urates. A large amount of calcific material was found in the larger joints, as well as at the commoner locations. The bizarre appearance of gout makes it desirable to consider this diagnosis in a case of painfully swollen joints.

Summary

A severe case of tophaceous gout is presented, together with x-ray and pathologic findings.

Mr. Ellis Rich of The Audiovisual Department assisted in the preparation of the reproductions.

Loma Linda Sanitarium and Hospital (Dr. Deeb).

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Role of Salt and Renal Mass in Experimental Hypertension

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The induction of high blood pressure by increased salt consumption has been reported in the rat,[1-3] rabbit,[4] and chicken.[5] Meneely et al.[6] obtained hypertension in rats chronically fed diets rich in sodium chloride. Some of the animals developed severe renal disease associated with massive edema and a nephrotic syndrome. In another chronic study from this laboratory[7] it was shown that rats which drink 1% saline over a long period of time frequently develop hypertension, together with generalized vascular lesions and renal damage. The latter was not associated with the nephrotic syndrome.

The experiments described in this report were undertaken in order to (1) compare the effectiveness of salt and of reduction in renal mass, individually and combined, in producing hypertensive vascular disease, and (2) clarify some aspects of the pathogenesis of both salt hypertension and renal-ablation hypertension.

Methods

Young adult white male rats weighing from 140 to 180 gm. were used. These comprised one control group and three experimental groups as follows: (1) control (40 rats)—standard diet and tap water for drinking; (2) salt (30 rats)—standard diet and 1% saline for drinking; (3) nephrectomy-salt (36 rats)—one kidney removed, standard diet, and 1% saline for drinking; (4) nephrectomy-water (32 rats)—one kidney removed, standard diet, and tap water for drinking.

Except for occasional interval killing, the experimental animals were followed for the duration of life. The controls were killed at periods corresponding to the death of the experimental rats. Food* and drinking fluid were offered ad libitum. The animals differed only with respect to renal mass and the type of drinking fluid. All were housed and maintained in similar manner.

The weight was recorded every week. The plethysmographic technique was used to measure blood pressure. Control readings were taken during the week before experiment and once weekly thereafter. Pressures of 150 mm. Hg and over were regarded as hypertensive. At periodic intervals fluid intake, urinary output, and quantitative albuminuria (Esbach method) were measured over a 24-hour period in all rats by use of metabolism cages. The blood urea nitrogen was determined before experiment, at occasional intervals during life, and routinely at the time of death. Terminally, analysis was made in many animals for total serum protein, albumin-globulin ratio, serum cholesterol (method of Kingsley and Schaffert), sodium, and potassium (flame photometer). Autopsies were performed on all animals and microscopic sections prepared routinely of practically every organ except brain. The heart was weighed after the atria were excised and the blood was removed. Cardiac index was determined from the formula $\frac{\text{heart weight}}{\text{body weight}} \times 100$.

Results

Control Rats.—These ranged from 8 to 19½ months of age at the time of killing. They were in good condition and gained weight as expected during the course of the experiment. The only lesions observed frequently, i. e., in about half the animals, especially those over one year of age, were in the kidneys. The changes here were of the same type as those in the experimental rats. The glomeruli showed exudation of plasma into the capsular space, adhesions, hyalinization, and accumulation of fat. The corresponding tubules contained colloid casts. Tubular atrophy with interstitial fi-

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* Pellets supplied by Rockland Farms, New City, N. Y. Sodium content is 0.88%.

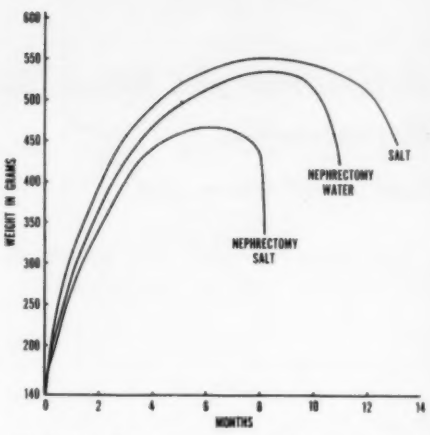
TABLE 1.—*Length of Life and Terminal Body Weight*

Rat Group	Life, Mo.		Weight, Gm.	
	Range	Mean	Range	Mean
Salt	7-16½	12	273-541	418
Nephrectomy-salt	5-10	8	218-540	356
Nephrectomy-water	7-16	11	318-580	400

brosis and lymphocytic exudate was present occasionally. As a rule the changes were merely focal and insignificant, although three animals had diffuse disease, diagnosed as nephrosclerosis. Two of these also showed generalized vascular disease, and one was perhaps hypertensive, even though the highest blood pressure was only 150 mm. Hg. This would constitute the only instance of spontaneous hypertensive vascular and renal disease observed in this laboratory so far. There was a total of three rats with spontaneous vascular lesions of the polyarteritis nodosa type, i. e., an incidence of about 7%, and these occurred at ages 14, 19, and 19½ months, respectively. Focal myocardial necrosis was found in only 2 of the 40 animals.

Experimental Rats.—Clinical Data: Length of life after onset of experiment is shown in Table 1. The life of animals having only one kidney and drinking saline was substantially shortened. Those with only one kidney and those with both kidneys but drinking saline lived longer than the nephrectomy-salt animals but less than the normal rats.

Typical weight curves for animals in each group are shown in the Figure. For about the first four months on experiment all animals gained weight fairly rapidly and at approximately the same increment. Thereafter the weight leveled off and then remained more or less stationary for a substantial period prior to a fairly sharp terminal fall. The weights of the salt and the nephrectomy-water rats were generally similar, although at corresponding intervals the former were usually slightly greater. The unilaterally nephrectomized rats which drank 1% saline were seriously stunted in



Typical weight curves for each experimental group.

growth and failed to gain weight after the fourth or fifth month. Only in rare instances did the maximum weight exceed 500 gm.

Daily fluid intake was variable but usually in the range of 20 to 50 cc. Polydipsia and polyuria were fairly frequent among nephrectomized rats on the salt regimen and infrequent among both the nephrectomy-water rats and those with kidneys intact but drinking saline instead of water.

The principal causes of death were uremia, intercurrent infection, especially pneumonia, or a combination of these.

Laboratory Data: Results are given in Table 2. Albuminuria, hypoproteinemia, and hypercholesteremia occurred fairly commonly, correlated well with renal damage, and were most frequent in the nephrectomy-salt group of animals. Next in frequency were the single nephrectomy rats on tap water, although the incidence here was only slightly greater than for the animals on saline. Loss of protein usually began several months after onset of the experiment and was then progressive. From initial (control) values of 0.02 to 0.08 gm., the quantity excreted over a 24-hour period was often in excess of 0.5 gm. and in several instances exceeded 1.0 gm. While the terminal values for serum protein usu-

TABLE 2.—*Laboratory Data*

Rat Group	Urinary Albumin, % with >0.5 Gm. Daily	Blood Chemistry, Range of Values *				
		Total Protein, Gm. %	Albumin, Gm. %	Cholesterol, Mg. %	Na, mEq.	K, mEq.
Control	8	4.8-6.4	2.8-3.6	25-48	140-151	3.9-7.3
Salt	32	5.1-7.2	1.7-2.5	32-141	132-174	4.8-9.0
Nephrectomy-salt	45	3.4-7.0	1.4-3.5	54-424	137-197	3.8-7.8
Nephrectomy-water	35	5.2-8.1	1.7-3.5	73-378	131-173	6.1-8.7

* All determinations made on serum.

ally ranged from normal to moderately reduced, the albumin fraction was low and the albumin-globulin ratio often less than 1. However, none of the animals was edematous. Terminal serum sodium was variable but generally within the normal range among the salt and nephrectomy-water animals. However, the levels appeared distinctly elevated in nephrectomized rats which drank saline. At the time of death several animals in each group showed an increase in serum potassium.

The blood urea nitrogen correlated fairly well with the degree of kidney damage. Terminal values for the salt rats with only one kidney were elevated almost uniformly and in 10 animals ranged from 140 to 360 mg. %. The unilaterally nephrectomized animals on tap water frequently showed a mild or moderate rise in blood urea nitrogen, and five had values from 100 to 224 mg. %. Over-all, renal function was least compromised among the rats drinking saline. However, half the group had at least a slight elevation of blood urea nitrogen and in three the values were in the vicinity of 100 mg. %.

Hypertension: Incidence is shown in Table 3. In most hypertensive animals the blood pressure usually ranged from 150 to 190 mm. Hg. The hypertension was labile at first, often remained so for a variable period, and subsequently became more sustained, especially in the latter part of the disease. Hypertension occurred in almost all of the salt rats with only one kidney. In several animals it was present as early as three or four weeks and was established in practically every instance within four months. Sixty-six per cent of the salt rats developed high blood pressure but as a rule not until they had been on the salt regimen for periods of from 6 to 12 months. Some animals had intermittent elevation in pressure to the 140-150 area during the course of the experiment but never became hypertensive. Hypertension was least frequent among the unilaterally nephrectomized animals on standard diet and water, i. e., incidence of only 16%. Here also the high blood pressure was a late event, usually developing only after the animals were on experiment for 8 to 14 months.

TABLE 3.—*Per Cent Incidence of Lesions*

Rat Group	Hypertension	Vascular Disease	Nephrosclerosis	Cardiac Lesions
Control	2.5	7	7	5
Salt	66	60	40	14
Nephrectomy-salt	94	83	74	60
Nephrectomy-water	16	45	56	16

Vascular Lesions: The incidence of generalized vascular disease closely paralleled that of hypertension in the salt and nephrectomy-salt groups. The frequency was greatest in the nephrectomy-salt group (83%), somewhat lower in the salt group (60%), and least in unilaterally nephrectomized animals on tap water (45%). Only in the last group did the incidence of vascular lesions exceed that of hypertension, i. e., 45% as compared to 16%.

Renal Disease: As with hypertension and vascular lesions, renal involvement was again most frequent and severe in unilaterally nephrectomized rats placed on a salt regimen (Table 3). The over-all incidence of kidney

TABLE 4.—*Weight of Heart* *

Rat Group	Cardiac Index		S. D. Diff.	Ratio Diff. of Means-S. D. Diff.
	Mean	S. D.		
Control	0.294	0.069		
Salt	0.422	0.098	0.025	5.2
Nephrectomy-salt	0.501	0.123	0.025	8.0
Nephrectomy-water	0.370	0.087	0.022	3.6

* S. D. indicates standard deviation; S. D. Diff., standard error of the difference between the means.

disease was 90%, and in most instances this was sufficiently marked to warrant a diagnosis of nephrosclerosis. Fifty-six per cent of unilaterally nephrectomized animals on tap water developed nephrosclerosis in the remaining kidney. However, the total incidence of renal disease, taking into account slight grades of damage, was 78%. Kidney lesions were least frequent in rats with intact kidneys and on the salt regimen. Sixty per cent of these had some renal involvement and forty per cent showed nephrosclerosis.

Cardiac Lesions: Myocardial damage was very common (incidence of 60%) in unilaterally nephrectomized rats drinking saline and relatively infrequent in the other two groups.

Cardiac Hypertrophy: Table 4 shows the mean cardiac index and its standard deviation in the control and experimental groups, the standard error of the difference between the means, and the ratio of the difference of the means to the standard error. The ratios are all greater than 2 and hence indicate a statistically valid increase in heart weight, especially for the salt rats with only one kidney and also for the salt rats with both kidneys. The data are consistent with cardiac hypertrophy.

Description of Lesions.—Vascular Lesions: These were usually widespread, involved arteries and arterioles, and occurred in the mesentery, pancreas, omentum, gastrointestinal tract, testis, liver, spleen, adrenal, kidney, heart, lymph nodes, and periaortic tissue. Lesions were commonly observed in the gross, especially in the mesentery, as nodules near the attachment of small bowel or along the mesenteric arteries. Microscopically, the disease was usually of the polyarteritis nodosa type. However, at times either one or two of the components, i. e., exudative, proliferative, or necrotizing, were poorly developed or absent, resulting in lesions such as polyarteritis without necrosis or pure fibrinoid necrosis. On occasion variability of lesions was evident in adjacent segments of the same vessel. Other changes consisted of thickening and edema of the media with enlargement and altered polarity of the muscle-cell nuclei, intimal hyalinization, and periadventitial fibrosis. The last two probably represented a chronic or healed stage of the disease.

Renal Disease: The renal lesions ranged in severity from slight to moderate or marked. In the former instance only a relatively small number of nephrons was involved and the kidney showed no significant gross change. When the renal lesion was severe and widespread, the kidneys grossly were pale and slightly enlarged and showed uniform granularity of the outer surface together with an indistinct cortical pattern. Thus the lesion closely resembled human nephrosclerosis.

Microscopically, the nephrosclerotic organs showed marked glomerular lesions consisting of exudation of plasma into Bowman's space, thickening of basement membrane, adhesions between tufts, slight epithelial and endothelial proliferation, hyalinization, and accumulation of fat. In addition there was fibrinoid necrosis of capillaries. Epithelial crescent formation was observed occasionally. Cellular exudate was either minimal or absent.

The tubules varied from atrophic to enlarged and were frequently the seat of fatty or hydropic degeneration. The lumens contained colloid casts, sometimes in enormous numbers, and very occasionally collections of polymorphs. Foci of interstitial fibrosis and lymphocytic exudate were fairly com-

mon. Pyelitis was observed in only a few instances, and hydronephrosis was essentially absent.

The small arteries and arterioles revealed fibrinoid necrosis either with or without accompanying exudative and/or proliferative components. However the latter in contradistinction to the vascular disease in the mesentery were rarely sufficiently well developed to warrant designating the lesions as polyarteritis nodosa.

Even without extensive interval killing it was possible to reconstruct the natural history of the renal lesions, since all grades of severity from slight to marked were observed among the animals. The glomeruli evidently constituted the primary site of disease. Very early this consisted of increased permeability of the capillary tufts as manifested by accumulation of plasma-like fluid in the glomerular spaces and in the lumens of the tubules. Then followed adhesions, progressive hyalinization, and accumulation of fat. In some instances fibrinoid necrosis of tufts appeared to occur early in the disease, and in others it followed the development of the other glomerular changes. Degenerative tubular lesions, also interstitial fibrosis and lymphocytic exudate, were interpreted essentially as secondary changes.

Vascular lesions commonly accompanied the glomerular disease, although they were observed on occasion when there was only slight glomerular alteration.

Cardiac Lesions: These were situated within the myocardium of both ventricles, especially the left, and were frequently subendocardial, although any portion of the myocardium might be involved and in several instances even the entire thickness of the wall. Grossly the lesions were sometimes visible through the pericardium as white nodules. Microscopically, there was necrosis of fibers of coagulative type, usually associated with interstitial fibroblastic proliferation and occasionally with fibrinoid necrosis of vessels. Either the necrotizing or the proliferative component of the lesion could predominate.

Comment

Chronic hypertension was readily produced in the rat by excessive intake of salt. The high blood pressure was associated with the development of diffuse vascular disease, nephrosclerosis, and cardiac hypertrophy and thus resembled human essential hypertension. Certain sequelae of human hypertension, i. e., congestive heart failure and cerebral hemorrhage, did not occur in the rat. In the latter chronic hypertensive vascular disease was usually terminated by intercurrent infection, uremia, or a combination of the two.

Salt was capable of inducing hypertension without any reduction in renal mass. However, the hypertensive effect was substantially enhanced by such reduction. Unilateral nephrectomy alone gave hypertension in only an occasional instance, but when this procedure was combined with increased salt intake hypertension occurred in practically every animal. There was an interesting dissociation between renal disease and high blood pressure among the unilaterally nephrectomized rats on tap water, i. e., 56% developed nephrosclerosis of the remaining kidney and only 16% had hypertension. The reason for this is obscure and the result contrary to what was anticipated in view of the long-term nature of the study.

The vascular lesions observed in this study were of the polyarteritis nodosa type. Despite the morphologic similarity, these should not be confused from the pathogenetic standpoint with human polyarteritis. The rat lesions are in all probability not of infectious or immunoallergic origin. Polyarteritis is the vascular change observed in the rat in association with experimental hypertension, regardless of whether the latter is induced by salt, renal injury, or renal ablation. Because of the necrotic component the lesion corresponds structurally to the vascular disease in human malignant hypertension. The latter usually consists of pure fibrinoid necrosis, although occasionally a small exudative component is present. The tendency of the rat to develop the poly-

arteric type of vascular change in connection with hypertension is unexplained, although it may represent a species reaction.

In spite of an extensive experience with young adult male rats in this laboratory, we have not yet observed a single instance of spontaneous polyarteritis nodosa among such animals. This corresponds to Wilens' study,[8] where the disease was limited essentially to old animals averaging 700 days at the time of death and was not observed before 500 days. He reported an over-all incidence among male rats of 3.5%, whereas a figure of 7% was obtained in the present study. The cause of the spontaneous disease is obscure, and a possible connection with renal disease, hypertension, and/or defect in salt metabolism has not been explored.

The myocardial necrosis in rats with hypertensive vascular disease in general resembles that obtained in rats with potassium deficiency.[9-12] In the latter state an electrolyte upset consisting of reduction in potassium content and increase in sodium occurs within the heart, and this may be the cause of the lesions. The degree of necrosis is significantly influenced by such factors as amount of salt intake [13,14] and acid-base balance.[15] Perhaps the mechanisms of cardiac injury in salt hypertension and potassium deficiency are similar, although at present this is largely speculative.

The renal disease in our animals would appear to be generally similar to that described by Meneely and associates [6] in rats given abundant salt in their food. However, their animals developed a full-blown nephrotic syndrome with massive edema, whereas in the present study there was albuminuria and hypoproteinemia but no edema. This difference in findings is not readily explained. Possibly less severe glomerular damage and proteinuria result from drinking salt, as in our animals, than from its ingestion in food. Sapirstein, Brandt, and Drury [1] suggested that in salt hypertension the ratio of salt to water intake was more important than the amount of salt.

All three groups of rats comprising this study showed the same type of renal disease, and this was designated as nephrosclerosis. While the suitability of this term may be controversial, we believe it is appropriate. Others have employed it in connection with steroid hypertension [16,17] and renal hypertension.[17] Its use inevitably invites comparison with the corresponding lesion in human essential hypertension, not only morphologically but also with regard to etiology and pathogenesis. Grossly the kidneys are typically granular and very similar in both rat and man.

Widespread glomerulitis [7] is a distinctive feature of rat nephrosclerosis. This undoubtedly accounts for the albuminuria, which is common in the rat but not in man. The glomerular disease resembles the benign form of human nephrosclerosis in some aspects, i. e., thickening of basement membrane, hyalinization and fusion of tufts, capsular adhesions, and fibrous obliteration and the malignant form in others, i. e., epithelial proliferation, crescent formation, and necrosis of tufts. Benign nephrosclerosis in man differs from the rat lesion in that it usually shows less conspicuous glomerular participation and a prominent vascular component of nonnecrotizing type, i. e., intimal hyalinization, elastic reduplication, and endothelial proliferation. The latter are in fact often poorly developed in the rat, where the vascular disease is mainly necrotizing. Hypertrophy and degenerative change of the medial coat of blood vessels occur in both human and rat disease, as do tubular atrophy, interstitial fibrosis, and lymphocytic exudate.

Rat nephrosclerosis cannot be as readily separated into benign and malignant forms as the human disease. This is mainly because the necrotizing vascular disease which accompanies high blood pressure in the rat has little or no connection with either the grade of hypertension or the degree of renal dysfunction. Structurally, the kidney damage in the rat resembles the malignant rather than the benign form of human nephrosclerosis.[16-18] The predilection of this animal to

develop diffuse glomerular disease and vascular necrosis may be a property of the species. It should be noted that these changes characterize the renal lesion associated with rat hypertension regardless of whether the latter is induced by renal injury, subtotal nephrectomy, or excessive salt intake.

The etiology and pathogenesis of human nephrosclerosis have not been established. It is commonly held that the renal disease rests primarily on a vascular basis,[19-22] i. e., arteriolar sclerosis, but if so the cause of the sclerosis is unknown. On the supposition that vascular disease is primary, the glomerular lesions have been attributed to ischemia, although thickening of the basement membrane of the capillary tufts constitutes a very early change.[22] Evidence for infectious participation is lacking. The role of hypertension in the induction of the vascular and glomerular changes is still not clearly defined.

More is known about the pathogenesis of rat nephrosclerosis, especially in connection with salt hypertension. From studies in this laboratory it appears likely that high blood pressure precedes significant morphologic damage to the kidney.[7] However, hypertension per se is not considered to play the main role in initiating either the glomerular or the vascular change. The early stage of glomerular disease may precede or develop simultaneously with arteriolar lesions. Later, as the glomerular damage becomes diffuse, the vascular disease appears inadequate in distribution and severity to account for it. As in man, there is no clear support for an infectious (including pyelonephritic) factor or for an immunoallergic mechanism. For this reason it would seem inappropriate to apply the terms nephritis or glomerulonephritis to the renal lesion as was done in studies on subtotal renal ablation.[23-25] The evidence suggests that both the glomerular and the vascular lesions in rats with salt hypertension result from the injurious effect of the salt itself.

Previous studies on subtotal renal ablation in the rat,[24,26,27] are pertinent to the present work. It was shown that removal of about 75% of kidney substance is followed by compensatory enlargement and then progressive disease in the remaining stump. Morphologically, there are glomerular, vascular, tubular, and interstitial lesions which result in a granular kidney. The latter, in its evolution and end-stage, is indistinguishable from the renal damage which we obtained in rats which drank saline instead of water, or in unilaterally nephrectomized rats on standard diet and water.

The hypothesis offered to explain the disease which develops in the renal stump following three-fourths nephrectomy is that when a small per cent of nephrons is forced to assume the entire burden of excretion, the functional overload is tolerated for a time in association with compensatory hypertrophy but eventually leads to decompensation and renal failure. Most vulnerable to the excess work load are the glomeruli, which undergo degenerative and proliferative change with gradual obliteration. Evidently the excretion of end-products of protein metabolism,[24,27-29] especially certain amino acids,[30] is particularly harmful. Thus the renal lesion following reduction in kidney mass is accelerated by high protein diet, especially animal protein, i. e., unilaterally nephrectomized rats on such a diet develop nephrosclerosis in about 6 months, as compared to 14 months for a standard diet. Also the lesion observed in the remaining kidney of unilaterally nephrectomized rats on high-protein intake is comparable in rapidity of development and severity to that in the stump of three-fourths nephrectomized animals on standard diet.

The present study affords convincing evidence that prolonged high-salt intake also has a deleterious effect on the kidney and promotes the development of nephrosclerosis. The renal damage from salt is readily obtained in intact animals and hence does not depend on reduction in renal mass. However, the injury to the kidney is clearly augmented when fewer nephrons are available for function. In rats with one kidney removed, nephrosclerosis of the opposite

organ occurs more rapidly and with greater frequency when saline replaces water for drinking.

It is important to reemphasize that focal lesions of early nephrosclerotic type often occur spontaneously in the rat, especially in the older age group, i. e., after 15 months. These may be associated with slight to moderate albuminuria. Diffuse renal disease with typical granular kidneys is apparently uncommon, only three examples having been encountered so far in this laboratory. Data on the incidence of spontaneous nephrosclerosis are now being obtained.

At present the origin of the spontaneous lesions is obscure. We feel that it is probably not on an infectious basis, including pyelonephritis, although the latter does occur spontaneously in the rat. Dietary insufficiency in early life is a possibility.[31] The observation that salt is a potent means of inducing nephrosclerosis may have some bearing. It raises the possibility that the spontaneous renal lesions result from diminished tolerance, inherent or acquired, for the salt consumed daily in the standard diet. Glomerular damage could then represent cumulative injury from dietary salt operating over a long period of time, or perhaps the glomeruli undergo change in relation to aging which renders them more susceptible to injury from salt even when daily intake is not excessive. Other dietary constituents, especially protein, might also be implicated in this way.

Unilateral nephrectomy in the rat is usually not followed by hypertension. None of the 10 rats observed by Rather[32] for 50 days after operation became hypertensive. In Loomis' group[33] of 11 unilaterally nephrectomized animals 2 were definitely hypertensive and 2 more questionably so. An incidence of 16% was obtained in the unilateral nephrectomy-water group of rats in this study, and a similar figure was reported by Grollman and Halpert.[31]

In contrast to one-half renal ablation, three-fourths ablation is commonly followed by hypertension.[26,34] The pathogenesis of the latter has not been satisfactorily explained. At first glance it seems consistent with Grollman's theory[35] that sufficient reduction in kidney mass results in loss of a renal secretory product which maintains normal blood pressure. However, this is somewhat difficult to reconcile with the interval of up to six or eight weeks which may elapse between nephrectomy and hypertension. To our knowledge no satisfactory evidence exists that three-fourths ablation results in the release of a pressor substance by the remaining stump. Nor can it be said that the hypertension depends on the prior development of lesions in the renal stump.[36]

Studies in this laboratory suggest a different basis for the hypertension following subtotal nephrectomy, and the following line of reasoning is offered in support. Excess salt readily induces high blood pressure, i. e., 66% of rats drinking saline become hypertensive, usually after a period of about nine months. Removal of one kidney raises the incidence of hypertension to over 90% and shortens the time required for its development to a few months. With three-fourths ablation the salt produces hypertension in practically all animals within one or two weeks. It seems clear that the greater the degree of renal ablation the more responsive the animal becomes to the hypertensive action of salt. Thus it appears plausible that rats with three-fourths ablation would be sensitive to average amounts of exogenous salt such as are present in the standard diet and that this might suffice to produce high blood pressure without the need for extra salt.

According to this concept the hypertension following subtotal nephrectomy is mediated by the toxic effect of salt, presumably without elaboration of a renal pressor substance, although we have no evidence for the absolute exclusion of the latter. Salt itself is more effective in leading to high blood pressure than is reduction in renal mass, at least at the 50% level. Another dietary component which may serve as a hypertensive agent in subtotal nephrectomized rats is protein,[27] although further documentation along this line is desirable.

From studies in this laboratory there appears to be a fairly well-defined pattern of events in the induction of hypertensive vascular disease by salt. The primary injury is to the peripheral blood vessels and the initial effect is evidently functional and consists of increased vascular tonus. This gives rise to hypertension, which thus represents a very early sign, perhaps the earliest, of widespread peripheral vascular involvement. At the time hypertension is initiated, the blood vessels are either morphologically unaltered or show only sparse and relatively slight lesions. The high blood pressure is accompanied after a variable interval by progressive diffuse structural damage to arteries and arterioles in such sites as pancreas, mesentery, adrenals, gastrointestinal tract, and kidneys. The kidney lesions, especially those in the blood vessels, develop more or less simultaneously with those of the other peripheral vessels and are regarded in essence as the renal component of the generalized vascular disease.

Thus the usual sequence in salt-induced hypertensive vascular disease is (1) increased tonus of the peripheral vessels, (2) hypertension, (3) generalized vascular lesions, and (4) nephrosclerosis. While the precise manner in which the vascular injury arises is not clear, it may well be related to electrolyte imbalances in the blood vessels initiated by retention of sodium and its subsequent accumulation within the vascular wall. Such a mechanism could apply to both functional and structural aspects of the vascular lesion. However, the delay in development of the morphologic damage suggests that there may be additional requisites for this component. There is some evidence that hypertension itself is not responsible for the vascular lesions.[37]

Further comment is in order on the role of the kidney in salt hypertension. Some grade of renal disease, ranging from focal vascular and glomerular change to well-developed nephrosclerosis, is almost invariably present in rats with chronic salt hypertension. In all probability such lesions do not initiate the high blood pressure, since at the onset of hypertension, and often during its early stage, the kidneys are essentially normal.

There is a parallel situation in man. Goldblatt [38] attributed essential hypertension to arterial and arteriolar sclerosis of the kidneys with the view that this caused liberation of a pressor substance from ischemic renal tissue. Yet here too there is evidence [19,39-43] that the high blood pressure commonly precedes the development of renal vascular disease and that in the early stage of hypertension arteriolar lesions are often minimal or absent. Although Moritz and Oldt [44] concluded that renal arteriolar sclerosis could be the cause of essential hypertension, their study was limited to patients with chronic hypertension and hence was not primarily designed to clarify the sequence of lesions. The thesis that organic renal vascular disease is responsible for essential hypertension in man is open to serious doubt.

While the kidneys do not play the primary role in initiating salt hypertension, they have an ancillary one related to their capacity to deal with salt and perhaps water.[45] Maintenance of electrolyte equilibrium within the body is an important aspect of renal function. One reason that some rats with intact kidneys remain normotensive even though on a long-term salt regimen may be that their kidneys are able to handle the excess salt in such a way as to prevent its accumulation in the tissues. Presumably the opposite effect occurs when renal mass is significantly reduced, since this tends to accent the defect which promotes retention of sodium. A similar situation might prevail in hypertension based on renal injury, since most techniques for the latter involve significant reduction in kidney mass or produce the equivalent effect by way of severe diminution in renal blood flow. Indeed, evidence that salt is a critical factor in the pathogenesis of renal hypertension in the rat has already been presented.[46] The nephrosclerotic process which develops in the remaining stump after subtotal nephrectomy does not per se cause hyperten-

sion. However, once the renal lesion becomes established, it very likely serves to sustain the high blood pressure and probably contributes to its irreversibility. This occurs by way of irrevocable loss of nephrons, with its attendant adverse effect on electrolyte management and hence on electrolyte distribution and composition in the tissues. The kidney disease which develops in rats with salt hypertension probably acts in the same way.

The studies dealing with the electrolyte composition of tissues in hypertension were summarized previously [46] and need not be recounted here. Interpretation of results has been hampered by shortcomings in method, especially in regard to intracellular and extracellular compartmenting of electrolytes. The data indicate a definite connection between concentration of tissue electrolytes and experimental hypertension but so far have not established a cause-and-effect relationship. The theoretical basis for supposing that an electrolyte upset in the peripheral vascular bed can give rise to increased vascular tonus and hence initiate hypertension was reviewed by Tobian.[47]

The critical item in salt hypertension is of course its relation to human essential hypertension. That the latter is influenced by salt seems certain from available data, and the possibility of a pathogenetic relationship cannot be excluded. From the pathologic standpoint there is much resemblance between the lesions of salt hypertension in the rat, i. e., diffuse vascular disease, nephrosclerosis, and cardiac involvement, and those of human essential hypertension. The natural history of the two diseases is similar. Over all, salt hypertension is much more like the human disease than is any other form of experimental hypertension, including the renal type. Thus there is a reasonable basis for the supposition that salt is an etiologic agent in essential hypertension.

Summary

Rats drinking salt water develop hypertension, together with generalized vascular lesions and renal disease. All these effects are enhanced by reduction in renal mass. Unilateral nephrectomy alone, although frequently followed by nephrosclerosis in the remaining kidney, does not result in hypertension. However, the latter develops almost constantly when salt is added to the drinking fluid. Renal-ablation hypertension may have the same basis as salt hypertension, and perhaps neither is dependent on the elaboration of renal hormones. It is postulated that the high blood pressure, as well as the vascular lesions and nephrosclerosis, arises through a similar mechanism initiated by the toxic action of the salt and may be based on disturbance in electrolyte equilibrium in the tissues, especially the vascular bed. Salt hypertension in the rat resembles human essential hypertension in its evolution and associated pathologic lesions. There is a distinct possibility that salt is one of the etiologic factors in essential hypertension.

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Ameloblastoma with Distant Metastases

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Introduction

Ameloblastomas are ordinarily relatively benign tumors with a tendency to recur locally and occasionally invade contiguous structures, but they rarely metastasize to distant sites. Although there are a number of reports in the literature dealing with distant metastases from ameloblastoma, only a few are well documented. We have considered as acceptable cases only those in which there was photomicrographic evidence that the tumor and its metastases were ameloblastoma. We have reviewed the literature published in the English language since 1880 on the subject of malignant alemoblastoma and found only three cases which met the above criteria. The purpose of this communication is to report a case of ameloblastoma of the mandible in which there was extensive invasion of the skull as well as metastases to lungs, pleura, and diaphragm.

Review of Literature

I. Acceptable Cases.—(*a*) Vorzimer and Perla [1] reported a 38-year-old man who had a history of a mandibular tumor removed at age 17. Six surgical procedures were carried out for the primary tumor and its recurrences. At autopsy there was a tumor "cast" in the bronchi of the right lower lobe of the lung with focal areas of infiltration.

(*b*) Schweitzer and Barnfield [2] reported a 34-year-old Negro woman who at age 23 had her first of 24 surgical procedures for a tumor of the mandible. At autopsy, 14 years later, there was tumor present in the maxilla, calvarium, and lungs.

(*c*) Simmons (Case 2)[3] reported a mandibular tumor in a 33-year-old woman who underwent four surgical attempts at removal of the tumor and its recurrences. Twelve years after the first appearance of the tumor she had a resection of one-half of the mandible and a radical neck dissection. Tumor was found in a lymph node at the carotid bifurcation which was identical with the tumor of the mandible.

II. Questionable Cases.—There were 14 reports of distant spread, but the secondary tumor was not confirmed by histologic examination or photomicrographs were not offered as proof. Eve,[4] Gentsch,[5] Havens,[6] Lozier,[7] Rattner (two cases),[8] and Catlin [9] reported secondary tumor in lymph nodes. Weisenfels [10] and Chont [11] both reported roentgenographic evidence of secondary ameloblastoma in the lungs without histologic proof. Simmons [3] mentioned that in Case 7 there was secondary tumor in lungs and lymph nodes but included no illustrations. New,[12] Spring,[13] and Coley [14] described secondary tumor in the submaxillary gland, in the frontal bone, and in the lungs, liver, and kidneys, respectively, but no photomicrographs were published. Kotanyi [15] referred to a malignant tumor with no further explanation.

III. Unacceptable Cases.—There were 20 cases which we were unwilling to accept as ameloblastoma. These fell into two general groups: 1. The primary tumor was interpreted by us on the basis of photomicrographs to be some other type of tumor, usually of minor salivary gland origin. 2. The metastatic tumor appeared as a sarcoma

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or squamous-cell carcinoma. These include cases reported by Ewing (two cases),[16] Heath,[17] Krompecher,[18] Papadimitriou,[19] Allgayer,[20] Murphy,[21] Jager,[22] Horsley,[23] Ch'in,[24] Villa,[25] Oinoue,[26] Phelps,[27] Watterworth,[28] Okinouye,[29] Grimes,[30] Behrman,[31] Sharp (two cases),[32] and Stoll.[33]

Report of a Case

History

A white woman first noted a swelling in the region of the angle of the left mandible in 1943, at age 18. Seven months later the mass had reached a size of 4 cm. in diameter, and surgical removal was undertaken at another hospital, in March, 1944, and again in May, 1944, when the tumor recurred. Although tissue was removed at each of these procedures and examined histologically, no tumor was seen.

The first of 23 admissions to the Presbyterian Hospital of Pittsburgh occurred in July, 1944, when roentgenograms of the left mandible revealed a 7 cm. cystic mass surrounded by a calcified capsule. This lesion had almost completely destroyed the ascending ramus of the mandible. This was carbolized, but no tissue was submitted for histologic examination. Subsequently, a purulent draining sinus developed in the left neck just below the left ear. An impacted, infected, left, third molar was removed in January, 1945. A painful swelling of the left jaw required incision and drainage in November, 1946. The sinus persisted intermittently, and in July, 1947, a mandibular exploration with curettement of the tumor resulted in the histologic diagnosis of ameloblastoma of the mandible. Review of the material from November, 1946, revealed a small fragment of tumor which represented the first histologic proof of ameloblastoma. At this time the tumor had extended from the angle to the condyle. Recurrent tumor was excised in October, 1948, and April, 1949. A left mandibular resection was performed in September, 1949, by which time tumor had extended into the pterygoid fossa. In January, 1950, a fat graft from the abdomen was placed in the left mandibular region for cosmetic purposes. By February, 1951, tumor had recurred in the mandibular stump and had extended into the maxilla, left antrum, nasal cavity, floor of the orbit, zygoma, and floor of the anterior cranial fossa. At this time the mandible was explored surgically and the anterior wall of the left maxilla was resected.

Five months later, eight years after the initial difficulty, the patient began having symptoms of paroxysmal nocturnal dyspnea and choking spells. Chest roentgenograms revealed scattered, well-defined, circular areas varying from 0.5 to 2.0 cm. in both lung fields. These did not involve the bones of the thorax. During the next six years radiation therapy of the tumor and the metastases was undertaken. The tumor of the left face responded favorably with a decrease in the size of the mass and much relief of pain. However, prior to any radiation therapy to the chest these tumors varied in size roentgenographically from time to time (Figs. 1 and 2). After two courses of radiation therapy to the chest the tumors in the right lung became much smaller and some were no longer evident. However, the tumors at the left apex and base continued to enlarge (Fig. 3). Because of extensive spread of tumor into the left orbit, the eye was enucleated in July, 1956.

The patient's last three admissions, in April, May, and August, 1957, were for protracted vomiting, dehydration, and terminally, disorientation and coma. The patient died on Aug. 17, 1957, at

Fig. 1.—Earliest chest roentgenogram, showing extensive tumor growth in both lung fields.

Fig. 2.—More advanced stage, four years later, prior to radiation therapy of the chest. Note variations in size of earlier lesions and appearance of new areas.

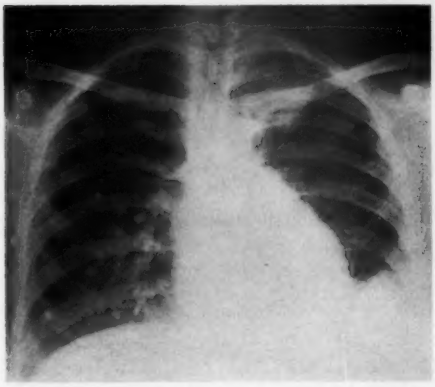

Fig. 3.—Four months prior to death, after three courses of radiation therapy. Majority of involvement in left apex and base. Note clearing of right lung.

age 32, fourteen years after the first appearance of tumor.

Autopsy.—Gross Description

A large tumor 12×9×3 cm. distorted the left side of the face. The skin over this area was mottled brown and ulcerated. Beneath the ulcer was a small abscess. The skin over the thorax was also pigmented. The left eye, mandible, and anterior portion of the left maxilla were absent. In these areas there was a moderately firm, lobulated, grayish-white tumor filling the left orbit and extending into the left maxillary, frontal sinuses, and left nasal cavity, through the nasal septum into the posterior right nasal cavity. Tumor extended through the floor of the anterior cranial fossa and encircled the optic nerves and sella turcica (Fig. 4). There was compression of the left frontal and temporal lobes and the pituitary, but no tumor was found within the brain substance.

Both lungs were involved with grayish-white cystic tumor. On the pleura of the left apex was a lobulated 8×6×4 cm. mass which extended superficially into the lung substance. A large tumor in the left base involved pleura, lung substance, and diaphragm. The tumor extended through the diaphragm and was adherent to the left lobe of the liver. This mass was 14×13×9 cm. and contained a 5 cm. cystic area partially filled with clear yellow fluid (Fig. 5). The remainder of the lung substance was reddish-gray, firm, and studded with several small, grayish-white nodules measuring up to 0.9 cm. in diameter. Similar nodules occurred over the right pleural surface and within the lung substance measuring up to 0.5 cm. in diameter (Fig. 6).

In addition there were bilateral renal vein thromboses. The remainder of the organs showed no significant changes.

Histologic Description

Except for minor variation, the histologic pattern of the tumor was the same in the many surgical specimens as well as in the several sites at autopsy. There were clusters, cords, and sheets of loosely arranged stellate cells surrounded by columnar epithelial cells (Fig. 7). Cysts of varying sizes and small clusters of squamous cells occu-

Fig. 4.—Floor of skull. Tumor replaces floor of left anterior and middle cranial fossae and the subdural tumor encircles the optic nerves and the pituitary; ×2/3.

Figure 5

Fig. 5.—Left lung, anterior surface. Involvement of apical and basilar portions with penetration of the diaphragm. Left lobe of liver adherent to tumor.

Figure 6

Fig. 6.—Right lung. Note tumor nodules beneath the diaphragmatic pleura and within the right upper and middle lobes.

pied the central areas (Fig. 8). Mitotic figures were occasionally present, and in some areas these numbered 1 per every 10 high-power fields. The amount and density of the fibrous stroma varied both in the surgical specimens and in different sites in

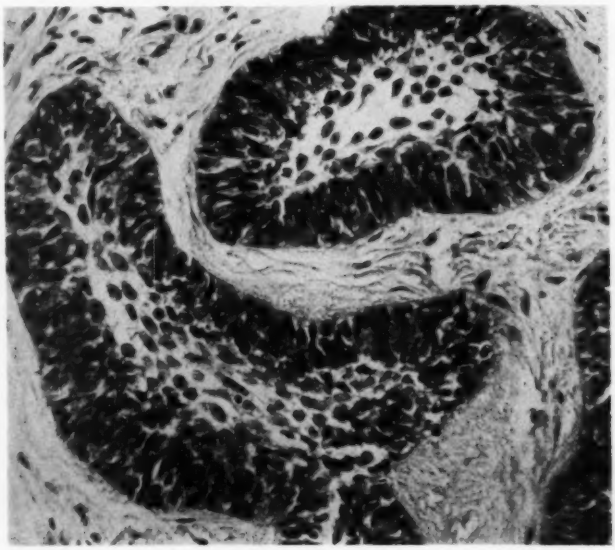

Fig. 7. — Tumor removed from mandible in 1949, showing the typical follicular arrangement of ameloblastoma having peripheral columnar epithelial cells surrounding central stellate cells; × 280.

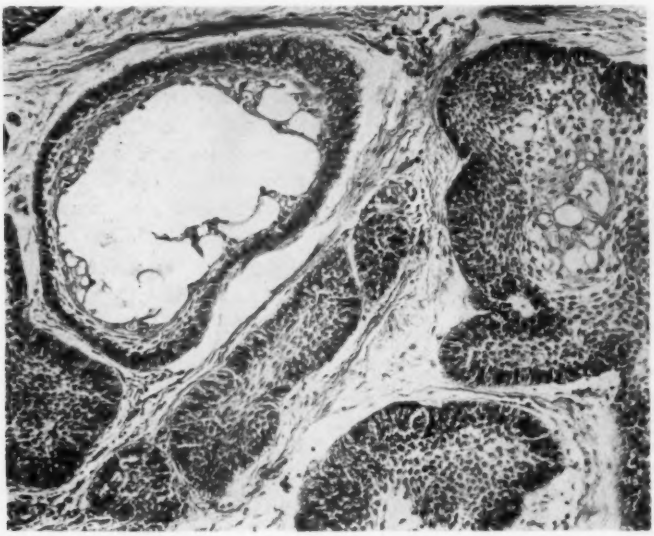

Fig. 8. — Tumor removed in 1951, showing central cystic and acanthomatous areas; × 140.

the autopsy material (Figs. 9 and 10). No definite relation between these stromal changes and radiotherapy was observed except for the lungs, where in many areas a dense collagenous stroma predominated. At the left base and apex of the lung, the stroma was dense collagenous tissue which compressed the epithelial cords and clusters. Calcified deposits were present in some of the more fibrous areas. A small amount of loose fibrous tissue separated sheets of epithelial and stellate cells in the tissue present in the cranial fossae (Fig. 11). No areas of anaplasia were seen in the tumor. There were calcified deposits within the connective tissue of the alveolar septa, the mucosa of the stomach, and some of the glomeruli, as well as in some of the smaller arteries of the spleen and kidneys.

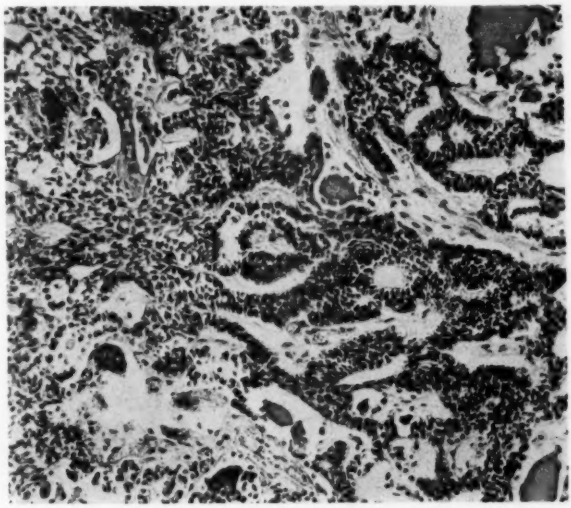

Fig. 9.—Right lung. Loose fibrous stroma separating typical cords of epithelial cells with plexiform arrangement, × 140.

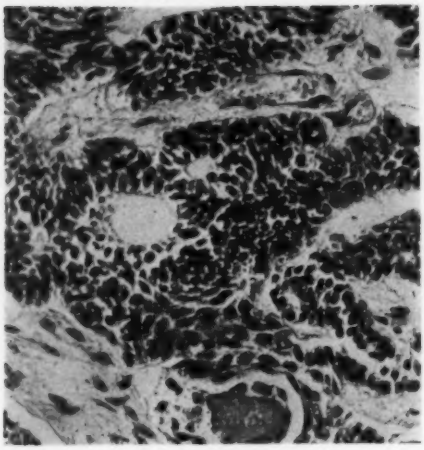

Fig. 10.—Higher power magnification of Figure 9.

Comment

The three cases in the literature which we have accepted and our case have certain features in common. They were typical ameloblastomas of the mandible of long duration with numerous recurrences and multiple operations. The secondary tumor had the same microscopic appearance as the primary. However, we found, as have others, that there were no significant histologic differences between this tumor and other ameloblastomas in our material that have pursued a benign course.

We are not able to resolve the question of how these tumors spread to the lung. The case of Vorzimer and Perla [1] is still apparently the only one in which an air-borne route appears reasonable. The large bronchial "cast" of tumor with associated bronchiectasis and lack of other nodules suggests that the tumor was aspirated. In our case as well, as in that of Schweitzer and Barnfield,[2] there was extensive invasion of the maxilla and base of the skull, as well as multiple nodules in the lung and pleura. They felt that the tumor in the lung was most likely blood-borne. That ameloblastomas may spread via the lymphatics is demonstrated by the case of Simmons,[3] and we have also seen it in one other case in this laboratory. It is of significance that no documented case of metastases to sites below the diaphragm have been reported. We have no proof of the mode of extension of tumor into the lungs in this case but believe it probably spread via the vascular system or perhaps was aspirated.

One other unusual feature of the case reported here is the remarkable variation in the roentgen appearance of the lung nodules both before and after radiation therapy. The nodules varied considerably in size during

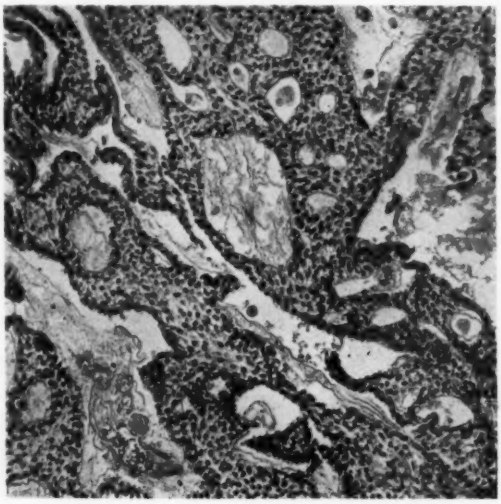

Fig. 11.—Intracranial tumor. Note the plexiform pattern with the peripherally arranged columnar cells; × 140.

the course of observation, and after roentgen therapy a large portion of the lung was practically cleared of tumor. A considerable degree of palliation was produced by the roentgen therapy to both the face and the lungs.

Summary

This report concerns a woman who developed an ameloblastoma of the mandible at age 18 and despite numerous attempts at local surgical removal died 14 years later. There was progressive local growth with extension of tumor to maxilla, antrum, orbit, and cranial cavity. Six years before death she developed numerous pulmonary metastases, which on two occasions responded remarkably well to roentgen therapy. Histologically no areas of anaplasia were seen, and it did not differ from other ameloblastomas which pursued a benign course. This case and the few other documented cases reviewed confirm the fact that ameloblastoma may rarely give rise to distant metastases in both lymph nodes and lung.

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Effect of Estrogens on Resolution of Local Cholesterol Implants

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The markedly higher incidence of death from coronary atherosclerosis in the white male as compared to the white female under the age of 50 in this part of the world is well documented.[1] This has been in part attributed to the possible effects of the estrogenic hormones. It is believed that estrogens may effect lipid metabolism or transport in the direction of antiatherogenesis. Whether this is a result of alterations produced by estrogens on serum cholesterol, phospholipid-cholesterol ratio, or shifts of β- to α-lipoproteins is not clear.

Little attention has been given to the possible direct influence of estrogenic substances on the removal of local tissue accumulations of cholesterol and various lipids. Stamler and his associates [2] noted that estrogens exerted a segmental antiatherogenetic effect in chickens with experimentally induced atherosclerosis. They noted that the estrogens exerted an inhibitory effect on the development of atherosclerosis in the coronary arteries but not in the aorta. In humans it has been noted that there are sex differences in the degree to which aortic athrosclerosis parallels coronary atherosclerosis. In the male under the age of 50 the degree of coronary atherosclerosis is usually greater than the degree of aortic atherosclerosis in any given person, whereas the reverse is the situation in the female.[3] It has also been demonstrated than an accumulation of lipid in the lymph nodes that drain diseased gallbladders is more marked in the young male with chronic cholecystitis and cholelithiasis than in the young female with similar disease.[4] This may in part be due to more rapid removal of the lipid from the lymph nodes in the female. For these reasons it was decided to investigate the possible direct action of estrogens on the resolution locally of cholesterol deposits in tissue. A nonvascular site was selected in order to eliminate hemodynamic influences.

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Department of Pathology, Beth-El Hospital.

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Material and Methods

In preliminary experiments with very large doses of estrone, 20 male rabbits and 20 Swiss albino male mice were used and divided according to weight and equivalent groups. The local implants consisted of small pieces of sterile absorbable gelatin (Gelfoam) sponge that had been saturated with known amounts of cholesterol in ether and in which the ether was allowed to evaporate. The gelatin sponge-cholesterol implants utilized in the rabbits weighed approximately 10 mg. each, and the implants used in the mice weighed approximately 2.5 mg. each. Fifty per cent of this weight consisted of cholesterol. All of the animals received these implants subcutaneously in the abdominal region. In the rabbits these were implanted through a small incision, whereas in the mice this was done with a tumor-transplant type of trochar. The rabbits each received four such implants and the mice one each. One group of animals received estrones in aqueous suspension by daily intramuscular injection for 35 days. The daily doses per animal were 1.0 mg. and 0.2 mg. for the rabbits and mice, respectively. The other group of animals received sham injections. All animals were housed and fed in a similar manner. Serum cholesterol determinations were performed on both groups of rabbits at the beginning, during the course, and at the termination of the experiment. At the conclusion of the experiment the local implant lesions were excised and examined grossly and fixed in formalin, and sections were prepared with hematoxylin and eosin and Sudan III.

After the preliminary study with the larger doses of estrone a more physiologic dose was used, one that would be comparable to dose levels re-

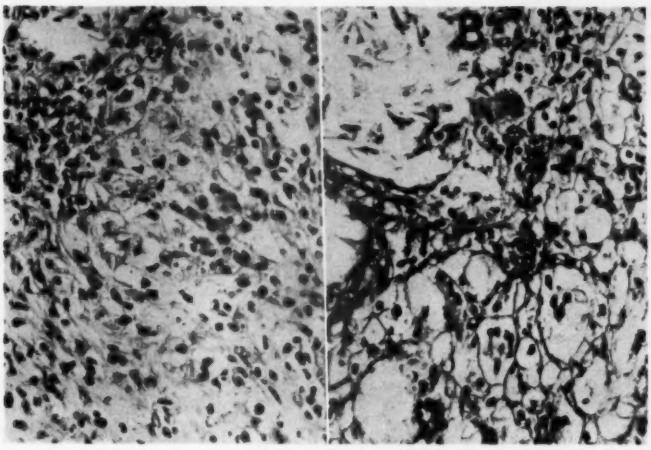

Fig. 1.—*A*, section of lesion from estrogen-treated rabbit, showing only a few vacuolated cells containing lipid. *B*, section of lesion from control rabbit, showing numerous cells distended with lipid and considerable extracellular lipid. Hematoxylin and eosin.

Fig. 2.—Entire representative lesion in mouse treated with 0.04 mg. of estrone at end of nine weeks. Lesion is small and reveals some remaining cholesterol clefts, some macrophages, and a few giant cells. Hematoxylin and eosin; reduced 10% from mag. × 135.

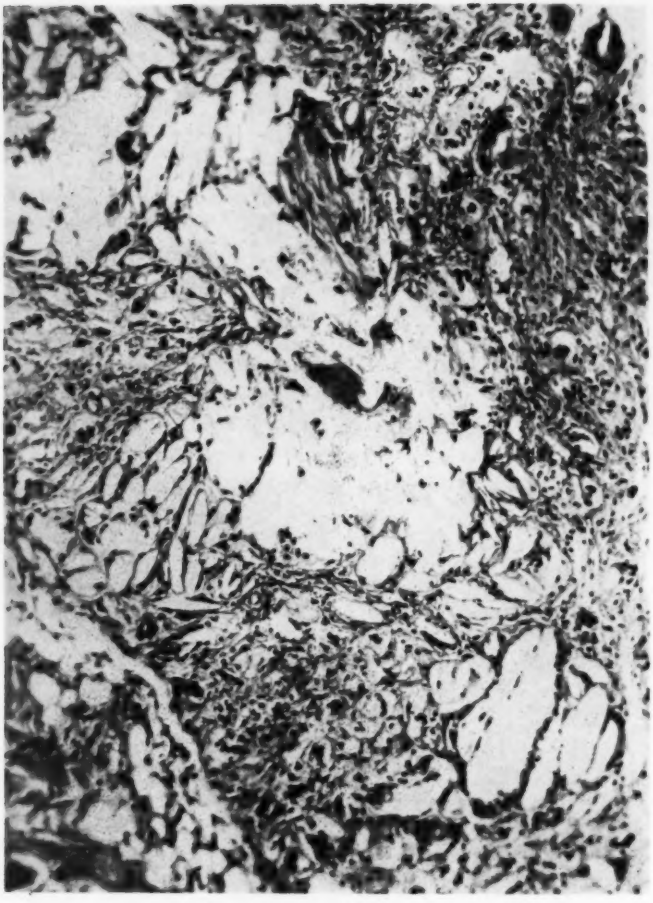

Fig. 3.—Entire representative lesion in nontreated mouse and of same magnification as Figure 2. Compared to lesion in Figure 2 it is much larger, with obviously more lipid. Hematoxylin and eosin; reduced 10% from mag. × 135.

quired for the production of estrus in the mouse. Only mice were used in this portion of the experiment. Four groups of Swiss male mice were each given implants and treated as follows:

Group A.—Thirty-six mice were given implants with absorbable gelatin sponges, each weighing 0.5 mg. and saturated with a solution of cholesterol, and were treated with 0.04 mg. of estrone intramuscularly every other day.

Group B.—Thirty-six mice were given implants with absorbable gelatin cholesterol sponges similar to Group A but received no treatment with estrone.

Group C.—Eighteen mice were given implants with absorbable gelatin sponge alone and treated with 0.04 mg. of estrone intramuscularly every other day.

Group D.—Eighteen mice were given implants with absorbable gelatin sponge alone and received no treatment.

One-third of the animals in each group were killed at three, six, and nine weeks after the initiation of the experiment.

Findings

In the estrogen-treated rabbits the gelatin-sponge-implant lesions were all grossly smaller than in the control group. In the mice receiving large doses of estrone, 8 out of 10 revealed complete resolution of the lesions and 2 revealed residual small lesions. In the comparable control group of mice the gelatin sponge-cholesterol implant lesions were grossly present in 9 out of 10 mice and were all larger than the two residual lesions in the treated mice. Histologically

the lesions in the estrogen-treated animals revealed at this stage a greater degree of fibrosis and fewer visible deposits of intracellular lipid or cholesterol. In the series of animals receiving the more physiologic doses of estrone the gelatin sponge-cholesterol implants at the three-week period revealed no significant differences as compared to the controls. However, by the sixth and ninth weeks greater degrees of resolution with significant decreases in the size of the lesions were noted in the estrone-treated animals as compared with the nontreated animals. In the animals with absorbable gelatin sponge implants alone, there were no significant differences between the treated and nontreated groups.

Comment

In this study it appears that estrogens in the form of estrone in aqueous suspension administered intramuscularly influenced the rate of resolution of the local gelatin sponge-cholesterol implants in nonhyperlipemic or noncholesteremic animals. Apparently this was not effected through any systemic alteration in serum cholesterol levels, since these levels as determined in the rabbits before, during, and at the termination of the experiment revealed no alterations. It has been previously reported that the reticuloendothelial system may play a role in cholesterol metabolism and that estrogens may stimulate the phagocytic activities of these reticuloendothelial cells.[5] Perhaps the enhanced resolution of these gelatin sponge-cholesterol implants under the influence of estrogens was facilitated through this latter mechanism. In studying the possible influence of estrogens on human atherosclerosis, consideration should be given to the possibility that local alterations may take place even in the absence of any evidence of systemic effects as manifested by serum cholesterol levels, phospholipid cholesterol ratio, or shifts in the lipoprotein pattern.

The absorbable gelatin sponge implantation technique furthermore lends itself as well to serial and semiquantitative studies of the influence of a variety of substances on the local resolution of not only cholesterol but various lipids and fats as well.

Summary

Intramuscularly administered estrone in rabbits and mice enhanced the resolution of local subcutaneous implants of absorbable gelatin (Gelfoam) sponge saturated with cholesterol. This occurred in the absence of any alterations in serum cholesterol levels.

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Effect of Necrosin on the Rate of Coagulation and the Implications Involved in Inflammation

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It has been shown that the euglobulin fraction particularly of acid exudates contains a factor termed necrosin,[1-3] which induces lymphatic blockade caused by fibrinous plugs or thrombi within lymphatic vessels. Vascular channels may be similarly affected with resulting thrombotic formation.[2-4] Obviously necrosin affects the clotting system, but the views of investigators differ considerably in regard to the exact role that this factor plays in the mechanism of coagulation. Some authors[5] assume that necrosin might perhaps be identical to thromboplastin; others have pointed out that the coagulating capacity of necrosin is similar to that of thrombin.[6] The present studies aim to elucidate this problem further. The observations substantiate with additional data the original finding of Menkin[1-3] that necrosin shortens the clotting time.

Material and Methods

Experiments were conducted on white male and female rabbits. The clotting time was determined at 37.5 C on 1.0 ml. samples of blood collected by heart puncture in siliconed tubes. The effect of necrosin on clotting time of blood was tested in vitro. The test was performed on 1.0 ml. samples of blood to which 0.1 ml. of necrosin was added. Two samples of necrosin, prepared from canine acid exudates according to Menkin's method,[4] were used. The effect on clotting time of increasing the concentration of necrosin in blood samples was also studied. By the method described by Quick, the prothrombin consumption time was likewise determined on the same blood samples.[7,8] Blood was collected from normal rabbits and in some cases from rabbits given intravenous injections of necrosin. Control experiments were conducted by using, in one series of animals, increasing concentrations of saline in blood samples and, in another group, increasing concentrations of euglobulin prepared from normal dog serum.

The thromboplastin effect of necrosin was studied on samples of oxalated rabbit plasma by use of Quick's one-stage method and substituting necrosin for rabbit-brain thromboplastin. Prothrombin and thrombin activity of necrosin was studied on tricalcium phosphate adsorbed rabbit plasma, with use of 0.1 ml. of deprothrombinized rabbit plasma, 0.1 ml. of 0.02 M $CaCl_2$ and 0.1 ml. of thromboplastin reagent, prepared from acetone-dehydrated rabbit brain.[7,8] An acetone extract of two samples of canine exudates was also tested for thromboplastic activity.

The effect of repeated injections intravenously of necrosin on clotting time, prothrombin time, and prothrombin consumption was studied on rabbits which received during the first 10 days 0.5 ml. of daily injections of necrosin, followed for several days by repeated injections of 1.0 ml. of the same substance at intervals of approximately 24 hours.

Several samples of freshly collected canine exudates at different pH were tested, and the effect of necrosin on the clotting time, prothrombin consumption, and prothrombin time was studied with use of 1.0 ml. of whole exudate and 1.0 ml. of oxalated exudate.

Necrosin has been shown by one of us (V. M.) to induce lymphatic blockade, so that material introduced at the site of necrosin injection remains fixed in situ.[3-4] To determine whether this effect may be referable to thromboplastin or thromboplastin-like material released by or contained in necrosin, thromboplastin obtained from acetone-dehydrated exudate by the method described by Quick for rabbit brain[7,8] was substituted for necrosin in an endeavor to study the effect on the

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patency of lymphatics. Two experiments were performed by injecting 56.8 mg. and about 85 mg., respectively, of thromboplastin in saline into the skin of the deltoid region of rabbits. On the following day 1.5 ml. of 1% trypan blue in saline was injected into the site, as well as into the corresponding site on the opposite forelimb to act as control. In one animal 1.5 ml. of saline without thromboplastin was injected into the control forelimb to ascertain that the saline per se of the thromboplastin-saline suspension was inconsequential. Cannulation under pentobarbital (Nembutal) anesthesia by micropipettes and study in the gross of the efferent tributary lymphatic vessels and nodes were performed by methods previously described.[9]

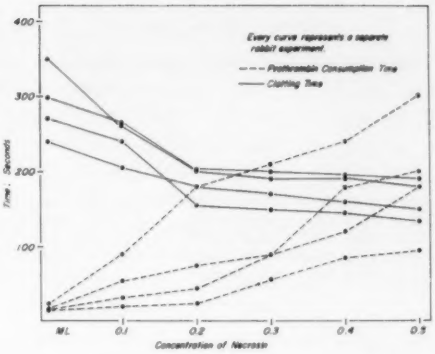
Finally, the repeated intravenous injection of necrosin probably induces the formation of a rabbit necrosin antiserum.[10] The in vivo neutralization of the injected necrosin in such immune animals doubtless would condition the results on clotting and prothrombin consumption time. The presence of precipitin antibodies to necrosin was determined by the usual technique after obtaining blood samples from the heart. Necrosin was diluted from 1:10 to 1:1,000,000 in saline and 0.1 ml. utilized for the precipitin test; 0.2 ml. of antiserum was employed for every dilution and the total volume brought up to 1 ml. with saline. After one hour incubation in a water bath at about 40 C the serological tubes were stored overnight in the refrigerator prior to taking readings. To rule out any nonspecific effect, the serum of a normal rabbit was tested serologically against diluted necrosin.

TABLE 1.—*Effect in Vitro of Necrosin on the Clotting Time and Prothrombin Consumption Time of Blood of Rabbits*

	Whole Blood		Whole Blood + Necrosin (10:1)	
Rabbit	Clotting Time, Sec.	Prothrombin-Consumption Time, Sec.	Clotting Time, Sec.	Prothrombin-Consumption Time, Sec.
Normal	270	14-16	150	52
Normal	260	12-14	140	80
Normal	270	10-12	170	65-68
Normal	480	14-15	150	93
Normal	240	14-15	150	120
Normal	300	19	150	80
55-35 *	270	18	240	65-67
55-36 *	300	26	210	67-70
55-35 †	270	18	240	55
55-36 †	360	16	265	90

* Samples of blood collected from rabbit after one intravenous injection of 0.5 ml. of necrosin.

† Samples of blood collected from rabbit after two intravenous injections of 0.5 ml. of necrosin.



Effect of necrosin on clotting time and prothrombin-consumption time.

Results

When 1.0 ml. of whole blood is mixed with 0.1 ml. of necrosin the clotting time becomes considerably shorter and the prothrombin consumption time is prolonged in blood samples collected from normal rabbits. The data bearing on these experiments are assembled in Table 1. Furthermore, the clotting time of necrosin-treated samples of blood collected from rabbits previously given intravenous injections of necrosin is not significantly altered, though the prothrombin-consumption time is somewhat high (Table 1). By increasing the concentration of necrosin added to blood samples obtained from normal rabbits, the clotting time is found to be correspondingly shortened while the prothrombin consumption time is prolonged. These changes are illustrated in the Figure. In brief, the effects on the rate of coagulation and on the prothrombin consumption time appear to be a function of the concentration of necrosin utilized (Figure).

Necrosin is contained in the euglobulin fraction of usually acid exudates.[1-3] As control for the effect found with this substance on the acceleration of the clotting time, the euglobulin of normal canine blood serum was obtained by utilizing the same procedure as employed for the extraction of necrosin from exudates, namely by $(NH_4)_2SO_4$ fractionation.[1-3] Increasing concentrations of the euglobulin of normal

TABLE 2.—*Effect of Increasing the Concentrations of Euglobulin Prepared from Normal Rabbit Serum on the Clotting Time and Prothrombin Consumption Time of Blood from Normal Rabbits*

Test	Clotting Time, Sec.				Prothrombin-Consumption Time, Sec.			
	I	II	III	IV	I	II	III	IV
1.0 ml. whole blood	240	360	255	270	38	28	22	32
1.0 ml. whole blood + 0.1 ml. euglob.	210	350	250	270	330	300	55	100
1.0 ml. whole blood + 0.2 ml. euglob.	210	350	230	270	No clotting		120	125
1.0 ml. whole blood + 0.3 ml. euglob.	210	350	215	270	No clotting		135	150
1.0 ml. whole blood + 0.4 ml. euglob.	210	350	215	270	No clotting		280 *	180 *
1.0 ml. whole blood + 0.5 ml. euglob.	210	350	215	270	No clotting		420 *	300 *

* Very faint clot.

serum induced in two out of four experiments no effect on the clotting time of whole blood. In two other experiments the clotting time was reduced only insignificantly. The results of these experiments are summarized in Table 2. When compared with the effects on the clotting time as shown in normal rabbits (Table 1) or in the Figure with the use of different concentrations of necrosin it is very apparent that necrosin hastens the rate of coagulation. When, however, the prothrombin consumption time is studied, a different picture is encountered, for in both instances the euglobulin of acid exudates (necrosin) and the euglobulin of normal blood serum display an appreciable increment (Tables 1 and 2).

If in place of necrosin or the euglobulin of blood serum, the final volume is maintained by substituting varying amounts of isotonic saline, it is very clear that, unlike the effect obtained with necrosin, the clotting time is not reduced but rather increased in the two experiments studied. These results are summarized in Table 3. Here again, however, the prothrombin-consumption time is enhanced by the addition of saline (Table 3).

TABLE 3.—*Effect of Increasing the Concentrations of Saline in Blood Samples on the Clotting Time and Prothrombin Consumption Time of the Blood of Rabbits*

Material	Clotting Time, Sec.		Prothrombin-Consumption Time, Sec.	
	I	II	I	II
1.0 ml. whole blood	165	240	27	20
1.0 ml. whole blood + 0.1 ml. saline	165	300	38	36.5
1.0 ml. whole blood + 0.2 ml. saline	180	390	38	36-38
1.0 ml. whole blood + 0.3 ml. saline	180	450	36	70
1.0 ml. whole blood + 0.4 ml. saline	220	455	38	65
1.0 ml. whole blood + 0.5 ml. saline	240	470	47	76-78

The foregoing results (Tables 1, 2, and 3) warrant the conclusion that necrosin accelerates the rate of coagulation of blood but that the effect on the prothrombin consumption time appears to be nonspecific. The effect of necrosin on coagulation confirms further, and now with the presentation of new data, the original observation by one of us (V. M.).[1-3]

Repeated intravenous daily injections of 0.5 ml. and then of 1.0 ml. of necrosin for even a few weeks do not influence significantly the clotting time, prothrombin time, and prothrombin-consumption time. Such observations were made on six rabbits, and the number of intravenous injections of necrosin ranged from 1 to 14. The data are conveniently collected in Table 4.

Thromboplastic effect of necrosin, tested on the same group of animals, is also represented in Table 4. Prothrombin time of the oxalated plasma determined according to Quick's original method remained essentially within physiological fluctuations.[7,8] Prothrombin time determined in the one-stage procedure, but by substituting 0.1 ml. of necrosin for 0.1 ml. of rabbit brain thromboplastin, is considerably prolonged (Table 4, Column 5).

TABLE 4.—*Effect of Intravenous Injections of Necrosin on the Clotting Time, Prothrombin Time, and Prothrombin-Consumption Time in Rabbit Blood*

Rabbit No.	Necrosin Injections, No.	Clotting Time, Sec.	Prothrombin Time, Sec.	Prothrombin Time, Sec. *	Prothrombin-Consumption Time, Sec.
1	1	450	14-15	--	8
2	2	330	14	--	8
3	1	300	7	--	21
4	1	270	8	--	19
3	1	220	7-8		
4	2	160	7	50	20
3	3	240	7	57-60	14
4	3	270	7	60	15
3	4	360	5	46-47	23
4	4	270	5	48-49	19
3	5	300	7	90	19
4	5	270	7	120	17
3	6	840	5	90	12 †
4	6	270	5	120	45
3	7	300	6	--	90
4	7	400	6	--	19
3	8	480	7	45	43
4	8	420	7	45	48
3	9	420	8	64-65	14
4	9	1,020	6	82-85	8 ‡
5	2	270	7	40-42	45
6	2	240	7	45-47	55
5	3	270	8	--	18
6	3	360	8	--	26
3	14	350	7	--	13
4	14	240	8	--	14
Control	--	300	7	75	19
Control	--	240	7	--	19

* 0.1 ml. of oxalated plasma; 0.1 ml. of necrosin; 0.1 ml. of $CaCl_2$ 0.02 M sol.

† In this experiment there was an interval of about 48 hours between the last injection of necrosin and the withdrawal of blood for the determinations.

‡ Determined 60 minutes after intravenous injection of necrosin; all other determinations on blood samples were performed at much shorter intervals after the injection of necrosin.

Clotting time is also appreciably increased when equal volume of necrosin is substituted for rabbit-brain thromboplastin in the prothrombin-consumption test. However, no clotting takes place when deprothrombinized rabbit plasma is tested with necrosin as a substitute for prothrombin or thrombin. This fact, shown by several observations, would not be quite in accord with the view expressed by Tanturi and his associates that the clotting capacity of necrosin is similar to that of thrombin.[6]

Thromboplastin prepared from canine pleural exudates treated with acetone, as in the original procedure described for the preparation of thromboplastin from rabbit brain,[7,8] displays considerably lower thromboplastic activity than rabbit-brain thromboplastin, as evidenced by the results obtained in the one-stage method. The results of these experiments are collected in Table 5. It is very clear that the clotting time, prothrombin time, and prothrombin-consumption time of exudates collected from dogs given injections intrapleurally of turpentine seem to be dependent on the pH of the exudate. When the pH of the exudate is at an alkaline level, prothrombin time and prothrombin-consumption time appear to be of the same order as those obtained with whole blood. However, as the pH decreases and the exudate becomes acid no clot is formed (Table 5). At this stage, this is probably referable to the fibrinous deposits in the

TABLE 5.—*Clotting Time, Prothrombin Time, and Prothrombin-Consumption Time of Canine Exudates*

Exp. No.	pH of Exudate	Clotting Time, Sec.	Prothrombin Time, Sec. *	Prothrombin-Consumption Time, Sec.	Prothrombin Time, Sec. †
1	7.51 †	No clotting	--	--	--
2	7.4	300	6-7	10-11	45
3	7.24	720	--	19	--
4	7.2	300	7	8-9	No clotting
5	7.0	900	Over 300	15-16	No clotting
6	6.8	900	Over 300	31-33	No clotting
7	6.7	No clotting	--	--	--
8	6.6	No clotting	--	--	--

* 0.1 ml. oxalated exudate; 0.1 ml. thromboplastin; 0.1 ml. $CaCl_2$, 0.02 M sol.

† 0.1 ml. oxalated exudate; 0.1 ml. necrosin; 0.1 ml. $CaCl_2$, 0.02 M sol.

‡ Exudate contaminated with blood.

TABLE 6.—*Effect in Vitro of Increasing Concentrations of Necrosin on the Clotting Time and Prothrombin Consumption Time of a Canine Exudate*

Exudate (pH 7.24)	Clotting Time, Sec.	Prothrombin-Consumption Time, Sec.
1.0 ml. exudate	720	19
1.0 ml. exudate + 0.1 ml. necrosin	450	19
1.0 ml. exudate + 0.2 ml. necrosin	450	19
1.0 ml. exudate + 0.3 ml. necrosin	450	20
1.0 ml. exudate + 0.4 ml. necrosin	420	24
1.0 ml. exudate + 0.5 ml. necrosin	420	25

inflamed area, thus leaving an exudate free of fibrinogen.

It is to be noted that when necrosin is added in varying amounts to an alkaline exudate the clotting time tends to be shortened and prothrombin-consumption time gradually becomes elevated in higher concentrations of necrosin. Such observations appear in Table 6.

Thromboplastin extracted from acetone-dehydrated acid exudate of a dog, unlike necrosin, is incapable when injected into the skin of a rabbit of inducing fixation of a vital dye such as trypan blue. The results of two experiments follow:

Rabbit No.	Interval Between Thromboplastin & Dye Injection, Hr. : Min.	Duration of Inflammation, Hr. : Min.	Thromboplastin Injected, Mg.	Dye Presence in Efferent Lymphatic Vessels & Tributary Nodes			
				Experimental Axillary Region		Control Axillary Region	
				Vessel	Node	Vessel	Node
52-47	18:55	20:33	56.8	++	+	+-++	+-++
52-46	18:44	20:35	85	++	+-++		+*

* 1.5 ml. of saline injected into skin of normal forelimb to control for suspension medium of thromboplastin.

The foregoing results indicate that exudate thromboplastin is incapable of inducing lymphatic blockade, for a dye readily diffuses into the tributary lymphatics. This evidence is apparently not in accord with the view expressed by some recent authors that necrosin and thromboplastin are perhaps identical substances,[5] for necrosin has been shown to induce marked lymphatic blockade, which prevents the diffusion of trypan blue into the tributary lymphatics.[1-3]

Earlier studies by one of us (V. M.) have revealed that repeated subcutaneous injections of necrosin into rabbits induce the formation of an antiserum to this euglobulin as detected by the precipitin reaction.[10] Nevertheless, a similar immunological experiment was again performed after the repeated intravenous administration of necrosin. The results obtained are tabulated below.

Rabbit 87 was given injections 20 times with 0.5 to 1 ml. of necrosin solution for 24 days; 4 days after the last injection it was bled from the heart (10 ml.); serum was obtained, and the precipitin reaction was performed against various dilutions of necrosin in saline.

Dilution of Necrosin	Presence of Necrosin
1:10	+++
1:100	++
1:1,000	+
1:10,000	Trace
1:100,000	?
1:1,000,000	0

The foregoing results confirm previous observations.[10] They indicate that necrosin is antigenic, yielding in the above experiment a titer of 1:10,000. This finding perhaps offers a reasonable explanation for the virtual absence of any significant effects on the shortening of the clotting time in rabbits repeatedly given injections intravenously with necrosin (Tables 1 and 4). The presence of antibodies to necrosin in the circulating blood would neutralize in vivo this substance and thus suppress its effect on the rate of coagulation. In two experiments (Rabbits 3 and 4, Table 4) after six and nine injections respectively, the prolonged clotting time may perhaps be referable, in part at least, to the lengthening of the interval between the last injection of necrosin and the withdrawal of the blood sample. The increased interval would possibly favor

more complete neutralization by antibodies of necrosin present in the circulating blood. When the above serological tests with varying dilutions of necrosin are performed against normal rabbit serum, no precipitin antibody is recovered attesting to the specificity of necrosin as an antigen.

Comment

The present studies confirm and extend Menkin's original observation [1-3] that necrosin accelerates the rate of coagulation of the exudate. The clotting time of rabbit blood treated with necrosin in vitro is dependent on the quantity of available necrosin, i. e., the higher the concentration of necrosin the shorter the coagulation time of the blood samples (Figure). According to Quick, the determination of the clotting time is a coarse screening test which is an unreliable method for estimating the decrease of prothrombin activity as induced by bishydroxycoumarin (Dicumarol) or some related drugs.[7,8] Nevertheless, in the present observations, necrosin as the euglobulin fraction of exudates is also compared in its effect on coagulation time with that caused by the euglobulin of blood serum. Since the same procedure is utilized in both cases, the shortening of the clotting time by necrosin, as the euglobulin of exudates, is believed to be real (Tables 1 and 2). Necrosin can be substituted for rabbit-brain thromboplastin in the one-stage method; but it cannot replace prothrombin or thrombin when used with rabbit deprothrombinized plasma. This fact seems to suggest that the role of necrosin in the coagulation system resembles somewhat that of thromboplastin. However, the thromboplastic activity of necrosin is not as potent as that shown by rabbit-brain thromboplastin. Furthermore, unlike necrosin, exudate thromboplastin appears to be incapable of inducing lymphatic blockade.

Necrosin seems to improve prothrombin consumption in vitro, but this does not appear to be specific (Tables 1, 2, and 3). Repeated intravenous injections of necrosin fail to influence the coagulation system, as evidenced by determinations of clotting time, prothrombin time, and prothrombin consumption time (Table 4). This finding may perhaps be related to the presence in the circulating blood of immunized rabbits of neutralizing antibodies to necrosin, as shown originally by Menkin.[10] Thus the demonstration of the antigenicity of necrosin is substantiated in our experiments.[10]

Freshly formed alkaline exudate tested by the one-stage method, prothrombin-consumption time, and clotting time show no essential difference (in at least two out of three experiments) from values obtained in normal blood (Tables 1 and 5). However, acid exudates do not show the same tendency to clot (Table 5).

The immunological basis of inflammation may be viewed as the ability of the inflammatory reaction to circumscribe an area of injury and thus fix in situ material injected into an inflamed area.[11] The primary mechanism involved is referable to the presence of thrombosed lymphatics and a fibrinous network at the site of inflammation.[12,13] In other words, the establishment of lymphatic blockade is of significance in evaluating the role of inflammation in immunity.[14] Furthermore, the rapidity and intensity whereby the tributary lymphatics are blocked serve as a gauge in determining the capacity for invasiveness by a micro-organism, provided that the latter happens to be the irritant involved.[15,16] In this way it has been pointed out by one of us (V. M.) that inflammation from the standpoint of immunity serves as the regulator of bacterial invasiveness.[14,4,11] Miles and Miles have recently raised objection to the view that lymphatic blockade is an important defense mechanism in inflammation.[17] Their studies have been conducted on mice and guinea pigs injected in the lymphatic plexus of the ear with a graphite suspension. The claim is based on the recovery of micro-organisms in areas of active inflammation away from the center of coagulated necrosis, where only lymphatic occlusion is admitted to occur. One of us (V. M.) has in the past pointed out that fixation is a relative phenomenon which must

be studied quantitatively. In other words, it is very essential to study not only the dissemination of micro-organisms either into tributary lymph nodes or into the systemic circulation but also at the same time their relative retention at the site of inflammation, as well as from a normal site of injection. This has been done in the case of Bacterium prodigiosum (Serratia marescens).[18] Such a study has not been performed by Miles and Miles.[17] Furthermore, when the peritoneal cavity of rabbits is utilized as the site of inflammation and from where drainage occurs through the substernal lymphatics, the studies with urea have clearly shown that dissolution of the lymphatic thrombi in rabbits eliminates the retention or fixation of graphite material in the inflamed peritoneal cavity.[19] These studies have indicated the importance of lymphatic occlusion as the primary mechanism involved, although other secondary mechanisms, such as adsorption or coagulation, in only certain instances, probably likewise play a significant role.[12-14] Finally, one of us (V. M) has often stressed that dissemination bears an inverse function to the intensity of induced local injury and a direct function to time (i. e., $D=\frac{t}{I}$).[14,4,11] It is therefore a question whether the interesting recent studies of Miles and Miles [17] on mice and guinea pigs essentially contradict the hypothesis that lymphatic blockade is an important defense mechanism, as earlier observations have shown with the use of different animals (rabbits and dogs) and at different sites.[4,9,11,12,15,18] The degree of induced local injury in the experiments of Miles and Miles [17] on mice and guinea pigs might have been entirely of a different order.

The fact that necrosin, liberated by injured cells at the site of an acute inflammation, hastens the rate of coagulation of both blood and exudates is obviously therefore of definite significance. The presence of this mediator would accelerate the "walling off" process in an inflamed area by the faster establishment of a lymphatic blockade in the form of thrombosed lymphatics and the formation of a fibrinous network in the acutely inflamed area. In this way, a definite immunological significance can be ascribed to necrosin, provided that its action in vivo is similar to the one displayed in vitro. This is supported by earlier observations which have already demonstrated that necrosin induces in the animal thrombotic obstruction to lymphatic drainage.[1-3]

Summary

Necrosin is a toxic euglobulin liberated by injured cells at the site of an acute inflammation. The presence of this mediator offers a reasonable explanation for the basic pattern of injury in inflammation.

Necrosin hastens in vitro the rate of blood coagulation and apparently also that of inflammatory exudates. The shortening of the clotting time of blood appears to be a function of the concentration of necrosin employed (Figure). The additional effect of necrosin in prolonging the prothrombin consumption time does not appear to be specific in nature, for a similar lengthening can be evoked with the euglobulin of normal blood serum or by addition of isotonic saline to blood.

The effect of necrosin in shortening the clotting time in vitro substantiates earlier studies.[1-3] This property of necrosin is discussed from the standpoint of inflammation as an immunological system. The presence of necrosin in an inflamed area would favor a more rapid circumscribing of the inflamed area, with the establishment of prompt lymphatic blockade by local thrombus formation and fibrinous deposits.

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The Pathogenesis of Hyaline Arteriolar Sclerosis

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There is no unanimity of opinion on the pathogenesis of the hyaline thickening found in the arterioles of the spleen, kidney, and many other organs, particularly in cases of hypertension. For the most part the lesion has been considered to be a degenerative one, but opinions have varied as to which part or layer of the arteriolar wall is the primary site of this degeneration.

Duguid and Anderson (1952), however, have suggested that the hyaline material, or its precursor, has been deposited on the intima of the vessel from the blood and has become incorporated in the wall; thus a process analogous to that of mural thrombosis and organization so commonly seen in larger arterial vessels, is occuring. This, they consider, is a more satisfactory explanation, compared with that of primary degeneration, for the final thickened and narrowed state of the vessel.

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While it is true that the precise nature of the hyaline material is a problem unlikely to be solved by histological methods alone, it is obviously important to ascertain whether in fact it could be hematogenous in origin, and this communication reviews the histological appearances of hyaline arteriolar sclerosis with this contention in mind.

Histology

Hyaline arteriolar sclerosis was studied in the spleen and kidney from forty cases of hypertension. In most cases only multiple random sections of both organs were examined, but in two cases used for teaching purposes, the opportunity was taken to study a large number of serial sections.

A low-power view of groups of these hyalinized arterioles is seen in Figures 1 and 2. These vary in appearance from what is commonly taken as the typical state of the vessels in this condition, a more or less uniformly thickened arteriole with a narrowed central lumen lined by plump en-

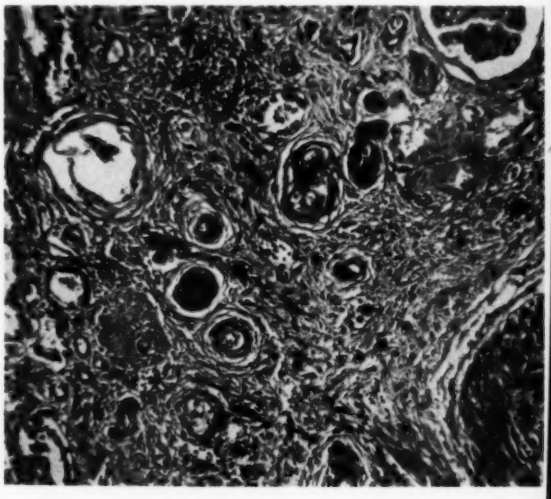

Fig. 1.—Low-power view, showing the variation in appearance of hyalinized vessels in the kidney. Hematoxylin and eosin; × 120.

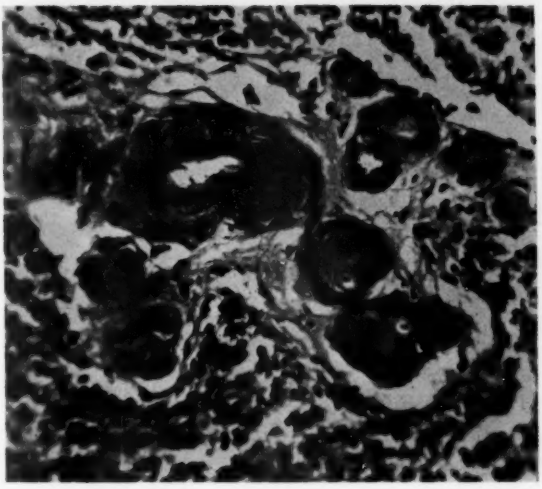

Fig. 2.—This shows a similar variation to Figure 1 in appearance in the spleen. Hematoxylin and eosin; × 180.

dothelial cells, to vessels which are completely occluded by hyaline material. Besides these there are a number of vessels which show eccentric thickening, some of it considerable. The vast majority of random sections of these organs show appearances like this, and for the most part the original arteriolar wall is unrecognizable save for a thin rim of cells externally.

The variation in form that these hyaline thickenings take is an aspect of the problem which has been somewhat neglected in the past. Yet they would appear to be of some significance, particularly those of complete occlusion and eccentric thickening. A study of a large number of the latter shows that very commonly the internal surface of the thickening has no endothelial lining and the rough ragged face of the hyaline material is directly exposed (Fig. 3), and this situation is just as often found in more concentrically thickened vessels (Fig. 4); moreover, it is very common to find these thickened vessels which lack an endothelial lining plugged by more recent eosinophilic granular material (Fig. 5). Appearances

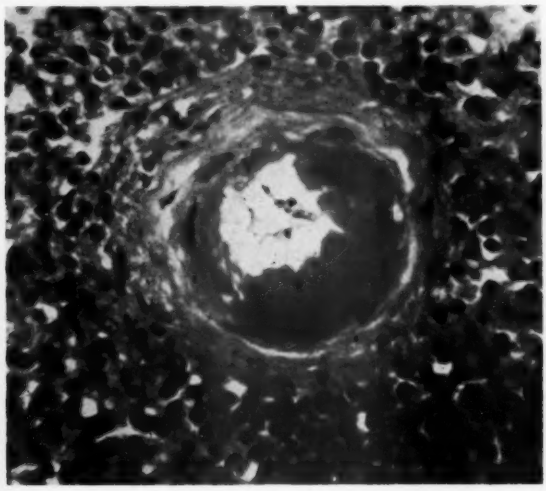

Fig. 3.—A splenic arteriole, showing rough hyaline material lining the inner wall and exposed to the blood stream. Hematoxylin and eosin; × 300.

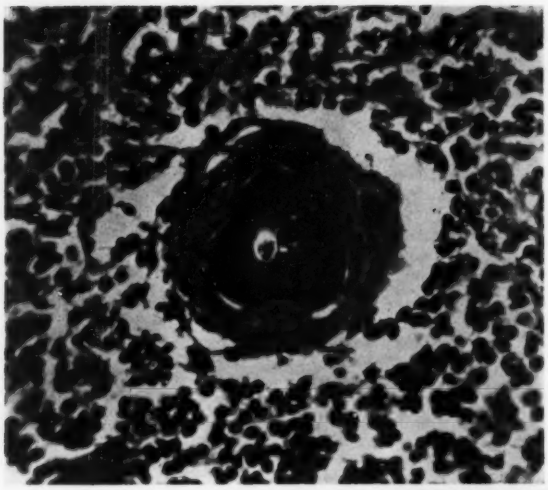

Fig. 4.—This concentrically thickened arteriole shows no evidence of an endothelial lining. Hematoxylin and eosin; × 300.

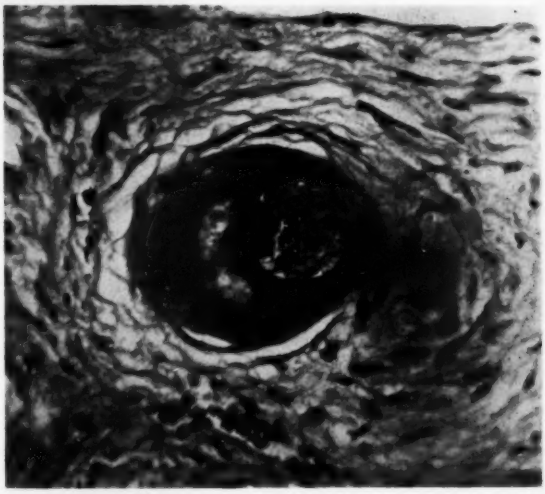

Fig. 5.—A hyalinized renal arteriole, showing eccentric thickening and recent plugging by eosinophilic granular material. Hematoxylin and eosin; × 300.

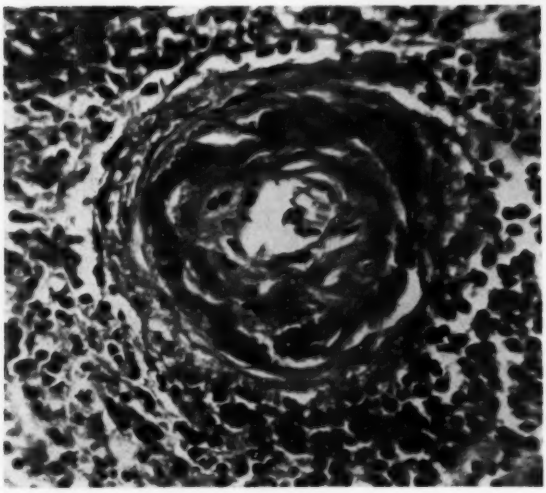

Fig. 6.—A splenic arteriole, showing a layered appearance which suggests repeated deposition of hyaline material. Hematoxylin and eosin; × 300.

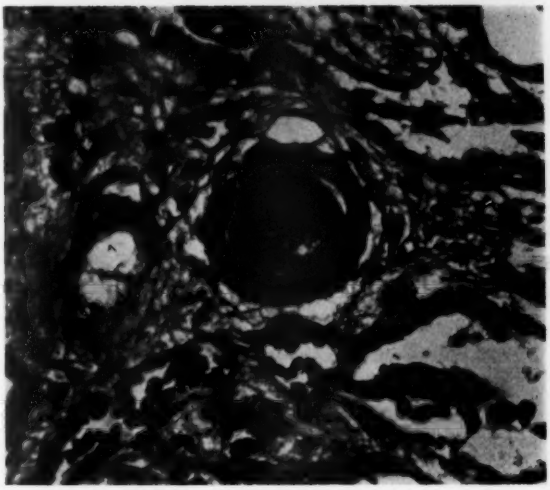

Fig. 7.—A renal arteriole completely occluded by hyaline material. Hematoxylin and eosin; × 300.

like these suggest that eccentric thickenings need not necessarily be primarily subendothelial in position as often supposed but may arise from surface deposits which become subendothelial by the overgrowth and readjustment of the endothelium. To substantiate this, there are vessels which show a series of endothelial linings sandwiched between a series of hyaline layers (Fig. 6), an appearance which suggests that episodic deposition and recovering has taken place.

The other very common appearance inviting attention is seen in those vessels which are completely occluded by hyaline material (Fig. 7). It would appear unlikely that occlusion like this is due merely to a degenerative process occurring in the vessel wall. Although in some cases, as in Figure 5, the plugging material appears fresher and more recent than that forming the thickening of the wall; in the majority no histological distinction can be made, and this applies also to the hyaline material often found ly-

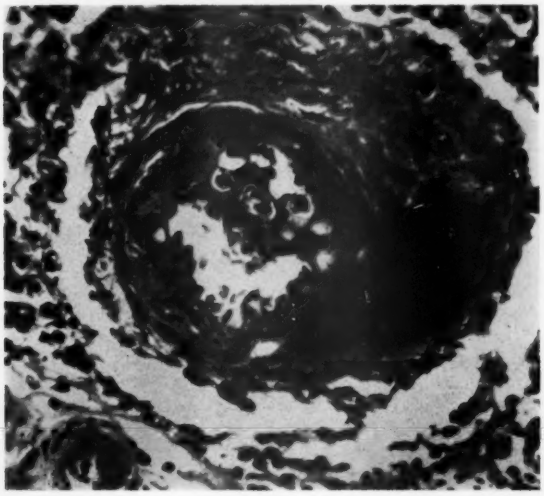

Fig. 8.—This splenic arteriole shows hyaline material apparently free in the lumen. Hematoxylin and eosin; × 300.

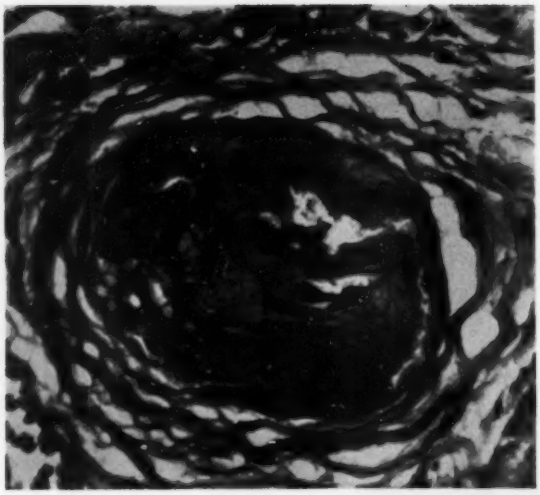

Fig. 9.—A larger renal arteriole, largely occluded, but showing two rudimentary spaces. Hematoxylin and eosin; × 300.

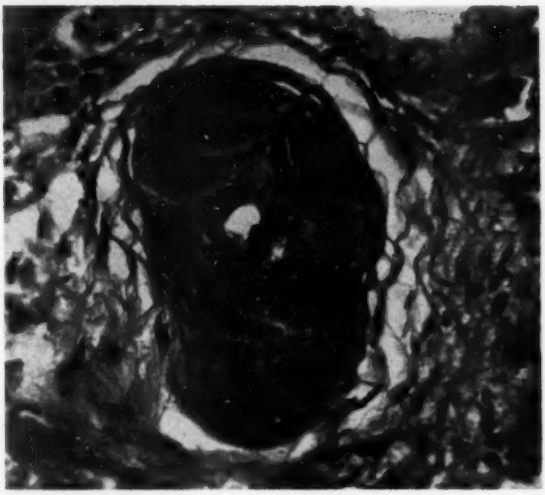

Fig. 10.—The same vessel as in Figure 9. A well-defined small central lumen is now present. Hematoxylin and eosin; × 300.

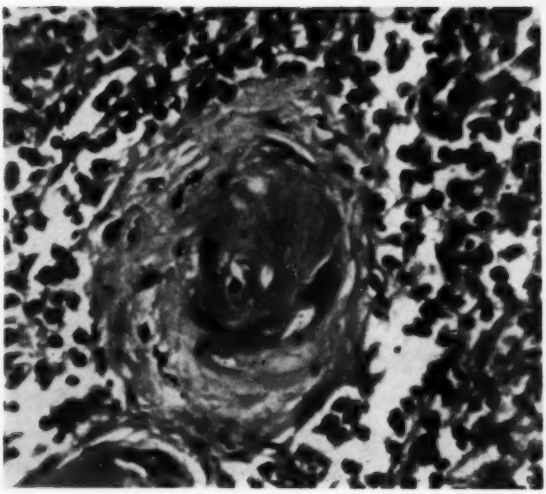

Fig. 11.—A splenic arteriole with a layered appearance and a central ragged lumen. Hematoxylin and eosin; × 300.

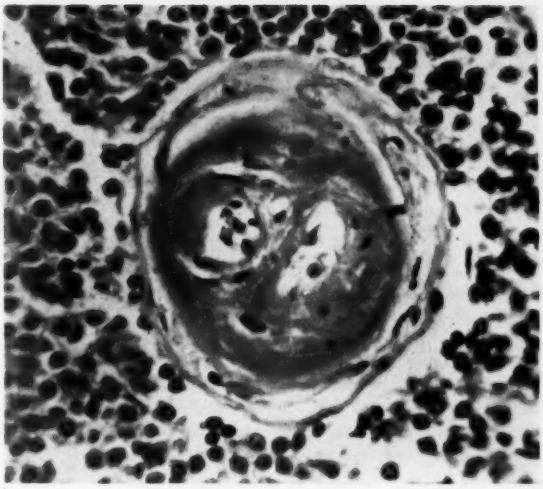

Fig. 12.—The same vessel as in Figure 11. Twin lumina are present, each with an endothelial lining. Hematoxylin and eosin; × 300.

ing apparently free in the vessel lumen (Fig. 8). Moreover, if these occluded vessels are followed by serial section, it soon becomes evident that complete occlusion is present only in a small segment and that a lumen of sorts is present or is re-formed either distally or proximally. This lumen is often rudimentary, sometimes central, sometimes eccentric in position (Figs. 9 and 11), but well-defined channels lined by endothelium are eventually formed, although considerable narrowing of the vessel remains (Figs. 10 and 12). These vessels present appearances very similar to those seen in the recanalizing of thrombosed arteries, and random sections of both kidney and spleen shown many vessels which give the same suggestive appearances (Fig. 13).

Comment and Conclusions

A review of hyaline arteriolar sclerosis by conventional histological methods reveals much presumptive evidence that the thickenings we have tacitly assumed to be due to an endogenous degenerative process could well be the result of exogenous deposition

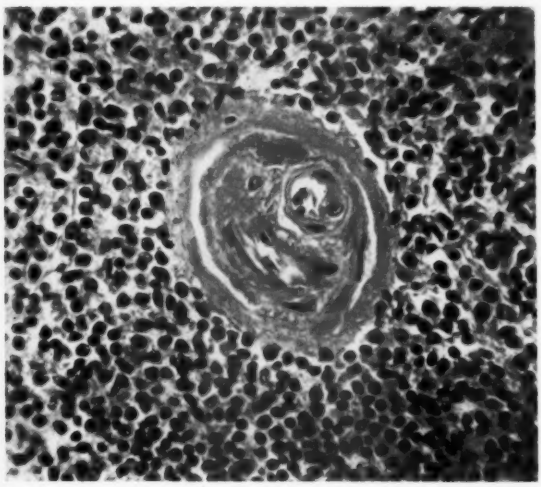

Fig. 13.—A splenic arteriole, showing appearances very suggestive of repeated deposition and probable recanalization. Hematoxylin and eosin; × 300.

on the vessel wall with subsequent incorporation. The widespread occurrence of vessels which are completely occluded and of vessels which show recanalization and the large number of arterioles showing eccentric thickenings, both with and without endothelial lining, makes it difficult to dispute the conclusions of Duguid and Anderson that we are seeing in these arterioles a process similar to occluding and mural thrombosis in arteries.

As these workers point out, it has always been difficult to justify the assumption that hyaline thickening was degenerative in character when faced with the physical fact of a vessel which appears extremely narrowed while still under some internal pressure. However, if we assume the process to be principally one of deposition on the vessel wall with a readjustment of the endothelium, narrowing becomes the logical result. It is also possible then to correlate the varying appearances of the vessel with the end-results of either occlusion and recanalization, which may be central or eccentric, or partial occlusion or mural deposition which results mainly in eccentric thickening; moreover, as we have seen, there is evidence to suggest that a recurring process of deposition and incorporation is taking place. Whatever the type of occlusion or silting up, it appears as if the endothelium and the arteriolar wall rearranges itself around the deposited material, and so it becomes successively subendothelial, medial, and eventually even adventitial in position.

Summary

The histological appearances of hyaline arteriolar sclerosis in the kidney and spleen are reviewed. These suggest that the hyaline material is deposited on the vessel wall from the blood then incorporated.

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Cardiovascular Factors in Development of Pulmonary Hyaline Membrane

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Many theories have been suggested to explain the frequent development of pulmonary hyaline membrane in the premature newborn.[9] The nonspecific nature of this lesion has been offered as evidence that the membrane is not a separate disease entity, and possibly of no significance per se.[7,10] Most of the emphasis in discussions on hyaline membrane has been on pulmonary factors.[9,10,14] Our observation, in a short series, of a consistent occurrence of hyaline membrane in cases of interatrial septal defect suggested a possible role for cardiovascular factors in the development of hyaline membrane. Accordingly, an analysis of a large series of premature newborn infants was done with the purpose of determining the influence of circulatory factors in the production of this lesion. Pulmonary capillary engorgement is a nearly universal finding in this group of infants, and extreme degrees of this change were interpreted as vascular congestion. This report deals with the lack of a direct relation between hyaline membrane and atelectasis or congestion, and with a positive relationship between pulmonary edema and hyaline membrane. The influences of heart size, malformations, and postnatal hemodynamic changes on the development of pulmonary hyaline membrane were studied and will be presented in detail.

Methods of Study

Materials and Limits of the Study.—All pertinent autopsy classifications in the files of the Department of Pathology, Duke Medical Center, for the period 1930-1955 were reviewed. Although some 934 cases were classed as premature, there were large numbers of premature stillborn infants classified as prematures, and this reduced the number of cases for further study to about 350. The analysis was restricted by limits on body weight and postnatal survival. According to Potter, hyaline membrane is generally limited to infants of between 1,000 and 2,500 gm. body weight. A large number of infants with birth weights between 500 and 1,000 gm. occurred in our series, and they are included in the data. The cases of all live-born prematures who weighed between 500 and 2,500 gm. and who lived more than one hour and less than 120 hours (5 days)were reviewed in detail. The autopsy protocols, clinical data, gross material, and tissue sections were examined. A series of 335 premature newborns is presented.

*Gross Examination of the Fetal Heart.**—To estimate the capacity of the ventricles in the fixed heart, a transverse cut is made across the ventricles parallel to the atrioventricular sulcus at just half the distance from the sulcus to the apex. The cross-sectional area at the level of this cut is estimated, and this is multiplied by the internal height of the ventricles as measured along the septal surface. This yields an estimate of the volumes of the fixed ventricles. When the heart is not fixed, it is possible to estimate more directly the capacity of the ventricles, but this method ruins the specimen for other purposes. The atria are trimmed to within 6-7 mm. of the sulcus. Blood is flushed from the ventricles through the atrioventricular valves, and residual water is removed with a small bulb pipet. A ligature is placed around the roots of the aorta and pulmonary artery. Holding the heart with the atrioventricular valves upward, water is added to each ventricle in turn until the valve leaflets float shut. This added volume we term the passive ventricular capacity. Then the ventricles are again emptied, and a small straight Kelly clamp is placed across the remaining atrial muscle as close to the valves as is possible without compressing the ventricles. Water is injected through the muscle into

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* A detailed description of the method of gross examination of the fetal heart used by the author in this study will be made available on request.

each ventricle in turn with a 26-gauge needle on a graduated syringe. In most cases, as the ventricle swells, a sense of resistance is felt. This is our end-point. Although no pressures were measured during these procedures, the right ventricle uncommonly continued to expand and burst at volumes rather large as compared with the other estimates. This volume we term the ventricular distensibility.

Criteria for Congenital Idiopathic Hypertrophy of the Heart.—There is a normal variation in the weights of organs for infants of the same body weight. Potter gives the mean heart weight of 4.6 gm. in a series of fetuses averaging 555 gm. body weight, and of 17.9 gm. in a series of fetuses averaging about 2,500 gm. body weight. What departure from the mean weight of a large series is to be taken as pathologic? A graph was constructed from the data of Potter to estimate the expected mean heart weight for fetuses classified by total body weight intervals of 100 gm. Her data yield a nearly straight-line relationship between body weight and heart weight for infants between 500 and 2,500 gm. Our study suggested that hearts weighing 1.5 or more times the mean for a given body weight class have an abnormal influence on postnatal hemodynamics, and for this reason all such hearts were considered to be hypertrophied. About 10% of the hearts in the 335 prematures studied were thus classified. Whether these represent a true entity or not could not be determined from our study. Because of this doubt, they are considered separately in the data on hyaline membrane.

Presentation of Data

Crude Incidences.—On histologic examination, 132 lungs from the 335 prematures showed hyaline membrane, an incidence of 39.4%. The incidence differed significantly when the data were arranged in groups, viz., section deliveries, cardiac hypertrophy, cardiac malformations, and controls. Table 1 shows this differential incidence. Each category was further analyzed by placing cases into groups of 500 gm. total body weight and by other considerations, to be developed below. The nonanomalous, nonhypertrophic, vaginally delivered infants constituted the control series. Table 2 gives the number of control infants in the four 500-gm. weight classes. The incidence for infants weighing less than 1,000 gm. is half that for infants weighing between 1,000 and 2,500 gm.

TABLE 1.—*Gross Distributive Incidence of Hyaline Membrane in 335 Premature Newborn Infants*

Class	Cases	Hyaline Membrane	Per Cent
Controls	256	75	29.3
Cardiac malformation	34	27	79.5
Idiopathic cardiac hypertrophy	34	22	65.0
Section delivery	11	8	73.0

TABLE 2.—*Incidence of Hyaline Membrane in Control Series by Body-Weight Classes*

Body-Weight Class, Gm.	Cases	Hyaline Membrane	Per Cent
500-1,000	82	14	17
1,001-1,500	95	34	35
1,501-2,000	55	19	35
2,001-2,500	23	8	35

Cardiac Malformations.—The commonest single malformation was isolated interatrial septal defect, which occurred 22 times. No distinction will be made between the varieties of this lesion which may occur.[1] There were six other hearts, classed with the above in the data, with interatrial septal defect combined with other lesions. These included two cases of tetralogy of Fallot and one each of pulmonary stenosis, left-heart atresia with wide ductus, proximal coarctation with patent ductus, and interventricular septal defect. In this total of 28 infants whose hearts showed interatrial septal defect, there were 23 lungs with hyaline membrane.

An extremely rare lesion, complete interatrial septum, as noted by Patten,[12] was seen twice. One case was classic, as described by Patten. The left heart was correctly formed, but was very small as compared with the right, the approximate left-to-right ventricular-capacity ratio being 0.25. The

TABLE 3.—*Incidence of Hyaline Membrane in Cases of Cardiac Malformation by Body-Weight Classes*

Body-Weight Class, Gm.	Cases	Hyaline Membrane	Per Cent
500-1,000	7	5	71.5
1,001-1,500	9	7	78.0
1,501-2,000	10	9	90.0
2,001-2,500	8	6	75.0

second case was not as striking. The septum primum was not fully adherent, there being one small point through which a probe could be passed. Both infants were immature, weighing 800 and 890 gm., respectively. The former lived 7 hours; the latter 24 hours. Both had hyaline membrane.

There were four other malformations. These were (*a*) interventricular septal defect with a normal foramen ovale, (*b*) tetralogy of Fallot with a normal foramen ovale, (*c*) isolated pulmonary atresia, and (*d*) proximal coarctation with a wide ductus. Of these, the first and last were associated with hyaline membrane. Table 3 shows the incidence of hyaline membrane in the cases of cardiac malformation by 500-gm. body weight classes.

Section Deliveries.—There were only 11 cases in this category, too small a number to justify division into 500-gm. weight classes. Of the 11 infants, 7 weighed less than 1,500 gm. and 4 of these, or 57%, had hyaline membrane. The comparable control series incidence is 27%. All four of the section infants weighing over 1,500 gm. showed hyaline membrane. This is in contrast to the 35% incidence for the comparable control group.

Cardiac Hypertrophy.—The 34 hearts classified as idiopathic hypertrophy had weights that ranged from 1.5 to 3.0 times the mean for that body weight. This range of values was split into two groups: those with heart weights 1.5 to 2.2 times the mean, and those with heart weights 2.3 to 3.0 times the mean. Table 4 gives the data for the former group. The incidences of hyaline membrane here are in the order of magnitude of those for section cases and malformations. Table 5 gives the same data for the latter group. Although this group is small, the incidences here are in the same order of magnitude as the control series. It should be noted that there is a greater proportion of smaller infants in this group, and that the control incidence is lower in the smaller infants.

TABLE 4.—*Incidence of Hyaline Membrane in Cases of Idiopathic Cardiac Hypertrophy with Heart Ratios of 1.5 to 2.2 by Body-Weight Classes*

Body-Weight Class, Gm.	Cases	Hyaline Membrane	Per Cent
500-1,000	3	3	100
1,001-1,500	8	5	63
1,501-2,000	9	8	89
2,001-2,500	3	3	100
All cases	23	19	83

TABLE 5.—*Incidence of Hyaline Membrane in Cases of Idiopathic Cardiac Hypertrophy with Heart Ratios of 2.3 to 3.0 by Body-Weight Classes*

Body-Weight Class, Gm.	Cases	Hyaline Membrane	Per Cent
500-1,000	5	1	20
1,001-1,500	5	2	40
1,501-2,000	1	0	0
2,001-2,500	0	--	--
All cases	11	3	27

Volumetric Estimations.—Measurements of the unfixed heart were made as described above to check the approximate range of values obtained from the review of fixed material. Representative cases are presented in Table 6. These measurements indicate that the right ventricle has a greater capacity. The data from measurements on 92 fixed hearts from the control series are noted in Table 7, and these also suggest a dominance of the right ventricle. No significant differences between the several weight classes and the presence or absence of hyaline membrane were noted.

Survival of the Premature Newborn.—Hyaline membrane was found in the lungs of one infant that lived just 5 minutes, and in the lungs of another infant that lived 20 days. The former infant weighed 1,500 gm.

TABLE 6.—*Representative Ventricular Volume Estimates*

Weight of Fetus, Gm.	Ventricle	Passive Capacity, Cc.	Distensibility, Cc.
750	Right	0.14	0.75
	Left	0.10	0.25
1,500	Right	0.6	4.2
	Left	1.1	4.0
1,750	Right	2.2	Not done
	Left	1.4	Not done
2,500	Right	2.2	4.2
	Left	1.4	2.8

TABLE 7.—*Left Ventricle-Right Ventricle Volume Ratios, from Estimated Capacities, in Cubic Centimeters*

Body-Weight Class, Gm.	No. of Hearts	Mean Ratio
500-1,000	18	0.72±0.04
1,001-1,500	41	0.81±0.04
1,501-2,000	25	0.72±0.02
2,001-2,500	8	0.74±0.06

and had a normal heart. The latter infant weighed 2,000 gm. at birth and showed a great-artery transposition with patent ductus on autopsy. Data on the 117 control infants weighing over 1,000 gm. were analyzed for survival. Those with hyaline membrane lived 15.9 hours on the average, and those without hyaline membrane lived 11.0 hours on the average.

Relationship of Other Pulmonary Findings to Hyaline Membrane in the Control Series.—Our study of the coincidence of vascular congestion, atelectasis, and edema with hyaline membrane in the control series yielded the observations summarized in Table 8. As may be noted, vascular congestion and atelectasis are nearly universal findings. Although hyaline membrane did not occur in any lung in the absence of either of these two findings, there is no apparent direct relationship between hyaline membrane and atelectasis or congestion. Edema was seen less frequently, and was found in only 132 out of 235 cases from the control series. It is significant, however, that all 71 cases of hyaline membrane found in this analysis were in this group of 132 infants. None of the 103 lungs without

TABLE 8.—*Incidence of Hyaline Membrane When Control Series is Analyzed for Various Pulmonary Findings*

	Lungs Positive for Finding			Lungs Negative for Finding	
Finding Evaluated	Cases	Hyaline Membrane	%	Cases	Hyaline Membrane
Congestion	227	72	31.6	8	0
Atelectasis	216	70	32.0	13	0
Edema	132	71	53.8	103	0
All controls	256	75	29.3	--	--

TABLE 9.—*Incidence of Hyaline Membrane When Cardiac Malformation Series Is Analyzed for Various Pulmonary Findings*

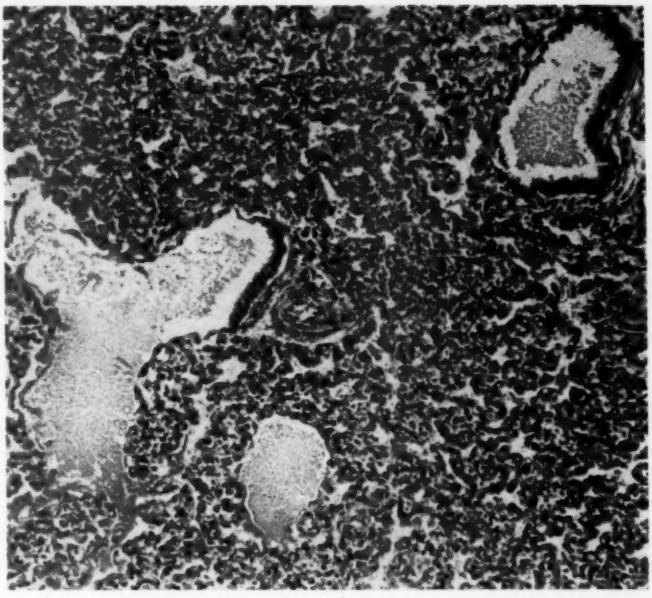
	Lungs Positive for Finding			Lungs Negative for Finding		
Finding Evaluated	Cases	Hyaline Membrane	%	Cases	Hyaline Membrane	%
Congestion	23	18	78.3	4	2	50
Atelectasis	29	23	79.3	5	4	80
Edema	29	27	93.2	5	0	0
All malformations	34	27	79.5	--	--	--

edema showed hyaline membrane. An intimate relation between edema and hyaline membrane is suggested by this observation.

Relationship of Other Pulmonary Findings to Hyaline Membrane in the Series of Malformations.—Table 9 summarizes the observations from a similar analysis of lungs from infants with malformed hearts. The significant feature here is that in the presence of a cardiac malformation, hyaline membrane was found in the absence of atelectasis and congestion, but not in the absence of edema.

Comment

The most striking feature of the lung containing hyaline membrane is the intense capillary engorgement, which we have interpreted as congestion. Potter especially emphasizes this point. From our own studies, atelectasis is a consistent feature, although many lungs showed only focal atelectasis. Another frequent finding is edema. That this is true edema, and not amniotic fluid, is strongly suggested by the recent study by Duran-Jorda et al.[4] The lack of amniotic debris is against aspiration of significant amounts of this material. There were but three lungs in this series with amniotic debris, and none of these showed hyaline membrane. The protein-bearing fluid in the air spaces has a granular or floccular character. This, we feel, is due to the fibrin present in the fluid.[5] Occasionally one finds a focus of fluid which stains more uniformly, like adult edema fluid, which, however, rarely contains fibrin or fibrinogen.



Photomicrograph showing extensive atelectasis and edema in bronchioles. In the overdistended bronchiole to the left there is a steady graduation in the density of edema, at the upper level, to hyaline membrane, at the lower level. Reduced to 80% of mag. × 175.

In some cases there is an increase in the compactness of this floccular material into a "hyaline membrane" in the same air space (Figure). Findings of a minor nature in these lungs include hemorrhages and leukocyte infiltrations. Hemorrhages were found in 29 lungs from the entire series of 335 prematures, and 11 of these 29 lungs had hyaline membrane. Leukocyte infiltration was noted only a few times, and in one case was a frank pneumonia.

Source of the Hyaline Membrane.—Our observations suggest that hyaline membrane is more closely related to pulmonary edema than to any other lung finding. Possibly the rapid respiratory rate of these infants contributes to a significant loss of water vapor from the edema fluid, increasing the protein concentration to the point of precipitation. This might be an explanation for the absence of the membranes in the stillborn. It has been shown clinically that infants considered to have the membrane improve in an atmosphere with an increased humidity.[10] Halting water-vapor loss would prevent protein precipitation, thus allowing for the reabsorption of the fluid over a longer period of time, and possibly prevent the formation of hyaline membrane.

Relationship of Cardiac Function to Hyaline-Membrane Formation.—The marked increase in hyaline membrane in deaths due to cardiac malformation suggests heart failure as a factor in the development of this lesion. Certainly pulmonary edema is a frequent feature of left-heart failure in adults and older children. Why are prematures without malformations more prone to develop hyaline membrane than term infants? Our studies suggest the following mechanism: In the full-term newborn there is a fairly constant decrease in the total weight of the conceptus when the cord is severed. We have elected to use the total weights to approximate the blood volumes or vascular-bed capacities. The residual fetal weight, as computed from ratios of fetal and placental weights,[15] is largely between 84% and 89% of the total weight of the conceptus at term. The ratio for prematures is different.[16] Similar computations reveal that the residual fetal weight is generally between 71% and 81% of the total weight of the conceptus. As may be seen in Table 7, the

estimated left-ventricular capacity for prematures is between 72% and 80% of that of the right ventricle. Blood flowing from the right ventricle probably constitutes the major portion of fetal circulation to the placenta.[17] Severing the cord would thus effect a reduction in the work load largely of the right heart. The residual fetal mass bears a ratio similar to that of the left and right ventricular capacities. It would seem, therefore, that the reduction in total mass is into the normal-capacity range of the left heart. A given increment in blood transferred to the fetus is a proportionately greater load for the premature heart, and imbalance would be more likely in these fetuses. The right ventricle, moving a maximal load over the intrauterine circuits, has perforce had greater work to do; and, in a sense, the flooding of the lung fields is due, in the absence of cardiac malformations, to this "overly strong" chamber forcing blood into the pulmonary vascular bed. The left ventricle, requiring time to develop its full strength,[8] may fail if the imbalance is too great, although failure of this chamber per se is not a prerequisite to marked pulmonary congestion.

Influence of Particular Cardiac Abnormalities.—Most of the malformations involved the interatrial septum, and the majority of these were septal defects. A free passageway for significant blood flow from one atrium to another would increase the right ventricular flow.[18] This would lead to an increased pulmonary flow. In this situation we have a left ventricle which up to the time of delivery has been less tested by a circulatory load than those hearts with a normal foramen ovale. These factors would contribute to what would be a relative failure of the left heart.

Of the 34 infants, 7 with malformations did not have hyaline membrane. What heart lesions were represented in this group? Three had an isolated interatrial septal defect. One of these infants weighed 850 gm. Another weighed 1,200 gm., lived only five hours, and had pulmonary edema. Had the infant lived longer, it might have showed hyaline membrane. Of the remaining four infants, one showed a failure of development of the left heart. The heart was essentially a bilocular one, with a small residual left atrium, which communicated with the right by a wide septal defect. The ductus was widely patent. Another infant had a combination of proximal coarctation, wide ductus, and interatrial septal defect. One infant had a heart with tetralogy of Fallot and a normal foramen ovale. The final case was one of an isolated pulmonary atresia with normal foramen ovale. It can be argued that the pulmonary blood flow in each of last four cases of the seven without hyaline membrane would be less than normal. Thus congestion and edema would be less likely. In fact, only one of these four infants did have edema, and it was minimal. This was the infant whose heart showed interatrial septal defect with proximal coarctation. This infant weighed 850 gm. at birth and lived 48 hours. By our proposed relationship of edema to hyaline membrane, we should expect no hyaline membrane in such cases. If these four cases were set aside in a special group unlikely to show marked congestion and edema, there would be left 30 cases of cardiac malformation which would be expected to show edema and congestion, and 27, or 90%, of these showed hyaline membrane. Of the malformation cases with pulmonary edema, 93.2% had hyaline membrane.

It might seem that the lesion most likely to result in left-heart failure would be proximal coarctation. Only one case occurred in the series, and it did not show hyaline membrane. Four other infants were rejected during the review. Two of these weighed 1,800 gm. apiece, but lived six days. Two others died on the first day, but weighed over 2,500 gm. None of these four infants showed hyaline membrane, but all four had edema. All five cases of coarctation had a wide ductus. It would seem that the pressure relations at the insertion of the ductus in instances of proximal coarctation are such that the ductus cannot close satis-

factorily, thus ensuring the shunting to the aorta of right-ventricular outflow. Congestion of the lung would seem unlikely in such instances, despite obstruction to left-ventricular outflow.

Influence of Postnatal Changes of the Heart on Hyaline-Membrane Formation.—The relationship of the foramen ovale to other structures in the right atrium differs with the age of the fetus, and possibly the mode of closure of this structure may differ also. Fetuses weighing less than 1,000 gm. frequently have a prominent pair of muscular ridges which traverse the right posteroinferior inner surface of the right atrium from either side of the orifice of the inferior vena cava to either side of the foramen ovale, creating the so-called posterior atrium. As has been shown, the foramen closes slowly after birth.[11,13] As long as anatomic patency exists, the foramen is available as a right-to-left shunt.

The rarity of closed septum indicates the importance of the in utero shunt, as the blood flow would maintain the patency of the foramen. The small size of the left ventricle in our two cases indicates that shunting of blood flow during fetal life is necessary for the proper development of the left heart. As the ductus closes in these hearts, the entire right-ventricular output is forced into the lungs, and, with the weak left heart receiving the entire load, failure would readily ensue. Persistence of a functional shunt at the ductus would relieve the pulmonary fields of some of the load. In the normal foramen, an increase in the left atrial pressure would close the shunt, increasing right-ventricular flow. Potter has observed that the right atrium is especially prominent in infants with hyaline membrane, suggesting a closed foramen, as well as backward congestion of the right heart. The increased venous pressures in newborns with respiratory distress, recently reported by Carter et al., are of interest here. Their studies indicated that those with distress and higher venous pressures would survive. Those with distress and lower venous pressures would die, and four of their seven autopsied cases showed hyaline membrane. Keen was able to show that the flow across the foramen was proportional to the pressure applied on the content of the right atrium. Possibly the higher pressure reported by Carter et al. served to keep the foramen patent, and thus reduced pulmonary blood flow. No cardiac malformations were reported in their series.

The closure of the ductus is important here. With the ductus closed, the right-ventricle output is forced into the lung fields. As noted by Barcroft,[2] the total blood volumes contained in the lungs do not differ before and after birth; thus the rate of pulmonary flow is probably increased with closure of the ductus. The degree of shunting will determine the pulmonary flow, and possibly reduce to some extent the tendency toward congestion of the lungs. This would give the left ventricle more time to adapt to the full load. Possibly a lessened lung flow would be slower and the blood better oxygenated. Keen has shown that the left ventricle becomes dominant during the second week of extrauterine life, indicating a slow and progressive adaptation. If transient immediate postnatal changes are important, then any injury or adaptive phenomenon leading to flooding of the lung fields would increase the tendency toward plasma loss into the air spaces, e. g., a left-to-right shunt across the ductus.

Relationship of Cardiac Hypertrophy to Hyaline Membrane.—No attempt has been made to examine further the relation of idiopathic hypertrophy to hyaline membrane. A case was recently reported suggesting this relationship.[6] We note, however, that this infant was delivered by section, as well as having a heart of immense size. This association of section and cardiac hypertrophy occurred three times in our series, and all three infants had hyaline membrane. The low incidence of hyaline membrane in infants with cardiac ratios of 2.3 to 3.0 times the mean suggests a critical size for heart failure. Another explanation can be found in the number of very small infants in this group. The relations of the foramen ovale,

known to differ slightly in this group, may indicate a functional patency of the foramen in this weight class more marked than in infants closer to 2,500 gm., and thus less pulmonary blood flow. Patency of the foramen need not be continuous to be of significance in relieving excessive right-atrial engorgement.

Maturity of Fetal Lung and Hyaline Membrane.—Hyaline membrane was found in 17% of lungs from infants weighing less than 1,000 gm. and in 35% of lungs from infants weighing more than 1,000 gm. Both groups show an increase in the incidence of hyaline membrane in the presence of cardiac malformations. The increase in infants weighing less than 1,000 gm. is proportionately greater, and thus both weight classes have values in the same percentage range in the presence of malformations. It may be that the premature lung capillaries more readily allow plasma proteins to pass into air spaces in failure of the left heart, but the differences in maturity of the fetal lung would seem to be of secondary importance relative to changes in hemodynamics. The disparity in incidence in the several control groups does suggest that all factors are not alike, but this conceivably could represent differences in blood flow about the foramen ovale, as well as any intrinsic difference in the respiratory epithelium or pulmonary capillaries. The known greater resistance to hypoxia of the smaller infants may also be of importance here.[17]

Relationship of Section Delivery to Hyaline Membrane.—No explanation of the relation between section delivery and hyaline membrane is found in our data. The question, in our opinion, is whether the finding in section infants of a significant increase in the quantity of cerebrospinal fluid,[14] relative to vaginally delivered infants, is evidence for a state of generalized hyperhydration, giving rise to heart failure or pulmonary edema by the mechanism of hypervolemia.

Essential Lesion of the Premature Newborn.—It would seem from our studies that atelectasis is the essential primary lesion in these cases. Further, there appears to be no positive relationship between atelectasis and hyaline membrane. The superimposition of cardiac failure from shock, cardiovascular malformations, or hypervolemia would give rise to pulmonary edema, which our data show to have a positive relationship to hyaline membrane. Infants with edema that survive for a period of time would be likely to show the hyaline membrane. Considering hyaline membrane from this point of view, we would deny that the membrane has any etiologic role, and would consider it the probably irreversible end-stage of what is essentially cardiac failure. It would follow, therefore, that attention should be directed toward atelectasis as the principal lesion in the premature newborn, and to the study of and prevention, diagnosis, and treatment of incipient cardiac failure in the premature newborn.

Summary and Conclusions

An analysis of cardiovascular factors and pulmonary findings was done on a series of 335 premature newborns. Pulmonary hyaline membrane was significantly commoner in instances of cardiac malformation. The majority of malformations presented circumstances in which the pulmonary vascular bed would be flooded in the presence of what seems to be a physiologically deficient left ventricle, and 90% of these cases showed hyaline membrane. Several malformations presented opposing circumstances in which this was not likely, and no hyaline membrane was found in these infants. The incidence of hyaline membrane was increased in infants whose hearts were considered to be hypertrophied by the criteria developed from the study. Many factors, including relative ventricular capacities and the possibility of significant postnatal functional patency of the foramen ovale, seem to bear on the adjustments of the neonatal heart. Our data suggest that the margin of cardiac reserve in the premature, especially with reference to the left ventricle, is less than in the full-term infant, giving rise to an increased incidence of failure and hyaline

membrane in the absence of malformations of the heart. The membrane itself is considered to be the end-stage of a progressive development of pulmonary edema fluid, concentration, and precipitation, and loose adherence to the walls of air spaces.

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The Weight of the Human Heart

I. "Normal" Cases

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Many studies have been recorded on the subject of normal heart weight. Among nearly 30 reports collected from primary sources [1-5] and from references [1-6] the means of the total heart weight differed widely, from 280 gm.[7] to 389 gm.[8] for males and from 250 gm.[3] to 384 gm.[8] for females. Atrophic hearts averaged substantially less, 240 gm. for males and 206 gm. for females.[9]

In addition to this great divergence of the means, the very measure of total heart weight is of questionable merit because its comprehensive, i. e., nondiscriminating, character may obscure what alone is relevant, namely, the weight of the myocardium. Thus, a moderate increase in the muscle mass of a chamber, even though pathological, may be insufficient to raise the total heart weight above normal limits. Conversely, an abnormally high total heart weight may not at all reflect an increase of myocardial mass but merely an excessive amount of epicardial fat. Unless one is dealing, therefore, with very heavy specimens, separate measurements for muscle, epicardial fat, etc., are needed to differentiate, for example, between a 370 gm. heart that is normotrophic and a 370 gm. heart which is hypertrophic.

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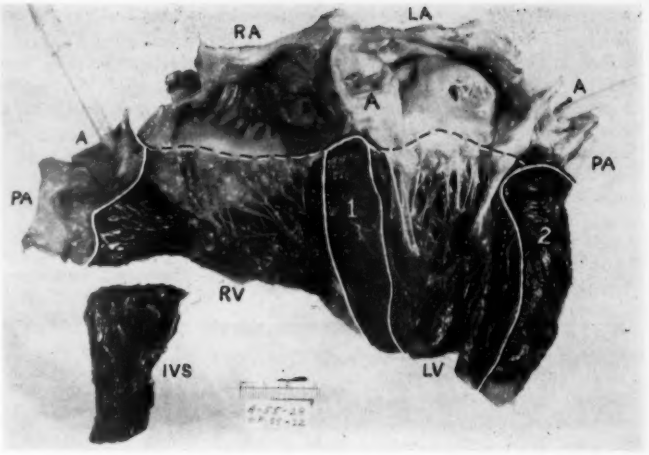
As much as one might wish for a single measurement in the diagnosis of cardiac normotrophy versus hypertrophy, there is unfortunately no procedural short cut for the classification of the individual heart. As will be shown, the time-honored practice of determining, also, the wall thickness of the chambers does not enlarge the diagnostic scope at postmortem examination. For one thing, the thickness varies with the tonus of the myocardium and its state of contraction or relaxation; second, the determination of thickness is inherently unreliable because of the uneven trabeculation of the chambers, and third, in a three-dimensional structure such as the heart, a linear measurement is much less sensitive an indicator of increase in mass than is the weight.

The present report, which deals with the "normal" heart, constitutes part of a larger biometric study undertaken (*a*) to review the possibilities and limitations of postmortem examination in the diagnosis of normal as against abnormal cardiac weights; (*b*) to analyze the factors, both physiological and pathological, which influence cardiac weights, and (*c*) to establish criteria of weight classification applicable to the individual heart.

We have adopted the principles of Müller,[5] which call for separate weight determination of the muscular and nonmuscular components. In addition to absolute values, such data furnish ratios among the various chambers which reflect both on their separate functions and on their mutual dependence.

Materials and Methods

The series comprised an unselected population of 292 adults, 21 years of age and over, that



Heart "unrolled" by the Schlesinger technique. Partitioning is carried out along the solid and dotted lines, as described in the text. The interventricular septum is composed of the free portion (in lower half of photograph) plus (*1*) the posterior insertion and (*2*) the anterior insertion. *A*, valve and root of aorta. *IVS*, interventricular septum. *LA*, left atrium. *LV*, left ventricle. *P*, valve and root of pulmonary artery. *RA*, right atrium. *RV*, right ventricle.

came to autopsy at Beth Israel Hospital, Boston, during the period 1953-1955. There were 160 men and 132 women. The cases may be considered representative of any mixed general hospital for the care of the acutely ill.

Preparation of Hearts.—A. Injection and Dissection of the Coronary Arteries: The hearts were removed by transection of the large body arteries and veins. The right and left coronary arteries were cannulated at their respective ostia and injected with a radiopaque mass, at 200 mm. Hg.[10] As soon as the mass had hardened, the heart was "unrolled" either by the original procedure of Schlesinger[11] or by a modification thereof.[12] X-ray arteriograms were complemented by dissection of the coronary arteries with scissors. Plaques, narrowings, occlusions, etc., were recorded by diagram and by description.

B. Dissection of the Epicardial Fat: The technique employed, as well as its practical and theoretical limitations, has been described previously.[13] In essence, the epicardial fat together with any contained coronary arteries (freed of injection mass) and veins were removed with scissors as completely as possible and weighed *in toto*.

C. Partitioning of the Heart (Fig. 1): After dissection of the epicardial fat, the atria together with the mitral and tricuspid valves were separated as a unit from the ventricles and the base of the interventricular septum. The free left and right ventricles were then freed from the interventricular septum at their respective anterior and posterior insertions. The roots of the aorta and pulmonary artery were removed at their respective valve rings.

Records.—Each case was recorded on a modified McBee card comprising, among others, the following entries.

1. Component Heart Weights: These weights included (*a*) free left ventricle (LV), free right ventricle (RV), interventricular septum (IVS), and (combined) atria; (*b*) epicardial fat, and (*c*) "excess," i. e., the roots and valves of the aorta and pulmonary artery.

2. Composite Heart Weights: The following weight combinations were calculated from the component weights: LV+IVS; total ventricular mass (LV+IVS+RV); total muscle mass (TMM), and total heart weight (THW=TMM+epicardial fat+excess).

3. Thickness Measurements: The data were based on the maximum thickness of LV, RV, and IVS as measured by various prosectors during the course of the study.

4. Myocardium: Fibrosis of the left ventricle and interventricular septum was assessed according to the area occupied by its surface projection and expressed as percentage of the combined projection area of LV+IVS. This two-dimensional quantitation was admittedly crude in that it took no account of the depth of fibrosis. Minor fibrosis demonstrable only under the microscope, as well as myocarditis and focal myocytolysis,[14] were recorded but not utilized as criteria for separate classification. There were no cases with myocarditis as the leading clinical or anatomic manifestation.

5. Coronary Artery Preponderance.[15]

6. Coronary Arteriosclerosis: Coronary arteriosclerosis was recorded in terms of degree and number of stenosing plaques. The degree of stenosis was estimated on a scale of from 0 to 4+; 1+ represented a reduction of the arterial lumen by no more than 25%; 2+ and 3+, a reduction of up to 50% and 75%, respectively, and 4+, a reduction of over 75%. Occlusions were listed as such.

7. Obesity-Leanness of the Body: This entry comprised (*a*) the state of alimentation as described in the clinical and autopsy protocols and (*b*) the maximum thickness of the subcutaneous panniculus adiposus of the abdomen as measured about the midline incision routinely used at autopsy. None of the bodies were weighed at autopsy.

8. Body Height: This measurement represented the crown-heel length as determined at the autopsy table. The series included seven patients (one man, 6 women) whose body height was not established either because both lower extremities had been amputated or because the measurement was not recorded.

9. Clinical and Postmortem Data: Included in this entry were (*a*) blood pressure, (*b*) pulmonary disease, (*c*) electrocardiogram, (*d*) diabetes mellitus, (*e*) congestive heart failure, (*f*) hemoglobin concentration, (*g*) eyegrounds, (*h*) clinical diagnoses, (*i*) gross and microscopic autopsy findings.

Terminology.—1. Left Ventricular Workload: To characterize the workload on the left ventricle the following data were taken into consideration:

(*a*) Blood pressure readings recorded during the terminal and all preceding hospital admissions. Unless clearly contradicted by subsequent readings, classification of normotension vs. hypertension was based on the blood pressure recorded at the time of admission, on the reasoning that this would reflect more adequately the tension under the stresses of daily life than pressures taken after confinement to bed. If there were more than one hospitalization the highest blood pressure of any of the admissions was used.

(*b*) Anamnesis. In addition to previous hospital records, the patient's personal knowledge of hypertension was taken into account.

(*c*) Clinical status. If a patient was admitted in shock from acute blood loss, myocardial infarction, etc., nonhypertensive blood pressure readings were regarded as "unknown" unless hypertension could be inferred from prior evidence. Cognizance was taken of conditions known to be conducive to left ventricular overload, such as chronic renal disease, arteriovenous aneurysm, Paget's disease of bone, or thyrotoxicosis.

On the basis of our analysis of partitioned heart weights and of certain cardiac ratios (see below), we have chosen for working purposes a blood pressure of 170/90 as the upper limit of normal.

2. Right Ventricular Workload: Because pulmonary arterial pressure had not been determined during life in any of the cases, the presence or absence of RV overload was inferred by correlating the weight of RV and the ratio (LV+IVS)/RV with the pathologic findings of the lungs upon postmortem examination.

3. Emaciation: A person was considered emaciated if a clinical or pathological diagnosis of cachexia had been recorded provided the thickness of the abdominal panniculus adiposus was not in excess of 1.5 cm. However, serous atrophy of the epicardial fat was viewed as equivalent to emaciation regardless of the thickness of the abdominal panniculus adiposus.

Normal Heart.—In order to establish "normal" weight ranges of LV and IVS the hearts of 45 cases were selected because they fulfilled the following criteria: (*a*) no myocardial pathology, (*b*) no valvular disease, (*c*) no left ventricular overload, (*d*) coronary arteriosclerosis no greater than 3+, (*e*) no emaciation.

These 45 cases comprised 26 men and 19 women. In none of the cases did coronary artery stenosis exceed 2+ except in two men, aged 59 and 77, and two women, aged 56 and 66, who had 3+ coronary artery stenosis.

There were an additional 14 cases fulfilling all the criteria of the above group except that they

TABLE 1.—*Age Distribution of Fifty-Nine "Normal" Cases Compared with Total Series*

	Age									
	Below 39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80 & Over	Total
Men											
Total series	4	8	6	14	14	25	27	33	15	14	160
"Normal," non-emaciated	1	0	2	2	4	5	2	2	4	4	26
"Normal," emaciated	1	1	0	0	0	1	2	0	0	1	6
Women											
Total series	9	4	7	14	15	16	26	13	18	10	132
"Normal," non-emaciated	6	1	3	0	2	2	5	0	0	0	19
"Normal," emaciated	1	1	1	2	2	1	0	0	0	0	8

TABLE 2.—*Distribution of Cases by Age, Body Height, and Abdominal Panniculus Adiposus*

Variable	No Emaciation						Emaciation			
	n *	Mean	Low	First Quartile	Median	Third Quartile	High	n *	Low	Median	High
Age, yr.											
Men	(26)	66	34	58	64	77	92	(6)	20	64	86
Women	(19)	50	21	36	49	65	68	(8)	31	52	64
Body height, cm.											
Men	(25)	167	150	164	168	170	178	(6)	162	168	172
Women	(18)	161	145	157	162	165	175	(7)	145	148	170
Panniculus adiposus, cm.											
Men	(23)	2.1	0.6	1.5	2.0	2.5	5.0	(6)	0.1	0.8	1.2
Women	(18)	3.0	1.0	2.4	3.0	3.7	5.0	(8)	0.2	1.0	2.8

* *n* indicates number of cases.

TABLE 3.—*Component Cardiac Weights* *

	Variate									
	L. Ventr.		R. Ventr.		I. V. Septum		Atria		Epic. Fat	
	M	F	M	F	M	F	M	F	M	F
No emaciation										
1. "Normal"										
n	20	15	14	14	20	15	17	19	20	16
Low	70	54	36	26	52	42	36	24	29	20
High	116	87	67	38	87	57	67	53	79	72
Range ratio **	1.7	1.6	1.9	1.5	1.7	1.4	1.9	2.2	2.7	3.6
Mean	94	67	46	33	64	40	49	39	61	50
Median	96	65	44	34	60	47	48	40	62	52
Sex ratio †	1.48		1.29		1.25		1.20		1.19	
2. Old age										
n			7 ‡				8 [5]		4 [6]	
Low			45				50		89	
High			58				63		107	
Median			49				55		96	
3. Right ventric. hypertrophy										
n			5 [1]	4 [3]			1 [6]			
Low			52	41			72			
High			142	89						
Median			60	46						
4. Excessive epicardial fat										
n				1 [7]					2 [4]	3 [4]
Low				41					95	103
High									128	192
5. Possible cutting error										
n	6	4			6	4				
Low	71	51			42	28				
High	114	82			67	46				
6. Total										
n	26	19	26	19	26	19	26	19	26	19
Low	70	51	36	26	42	28	36	24	29	20
High	116	87	142	89	87	57	72	53	128	192
Range ratio	1.7	1.7	3.9	3.4	2.1	2.0	2.0	2.2	4.4	9.6
Emaciation										
n	6	8	6	8	6	8	6	8	6	8
Low	67	36	34	14	42	30	31	16	14	4
High	97	72	66	32	70	50	60	48	91	48
Median	83	54	43	25	51	42	41	30	35	23

* In grams.

** Range ratio is $\frac{\text{High}}{\text{Low}}$.

† Sex ratio is $\frac{\text{Median of males}}{\text{Median of females}}$.

‡ Age groups are indicated as follows: [1] 77-92, [2] 75-92, [3] 81-92, [4] 46-67, [5] 25-66, [6] 51, [7] 56, [8] 56-64, [9] 56-66.

were emaciated, 6 men and 8 women. Coronary artery stenosis was 2+ or less except in 3 women between the ages of 56 and 66 who had 3+ narrowing.

The age distribution of the entire series is being presented in Table 1 and contrasted with the "normal" cases that form the topic of the current discussion.

Age, body height, and obesity-leanness (thickness of abdominal panniculus adiposus) of these 59 cases are recorded in Table 2, the distribution being identifiable by combined reference to the means, the range and the first quartile, median, and third quartile of the array.*

In Tables 3-5 are listed the component heart weights, the composite heart weights, and the wall thicknesses, respectively.

In Table 6 are presented the ratios of component and composite heart weights. The cases are subdivided whenever their weights or ratios appear modified by RV hypertrophy, old age, excessive epicardial fat, or cutting error.

* First quartile: Case $\frac{n+1}{4}$. Median: Case $\frac{n+1}{2}$. Third quartile: Case $\frac{3(n+1)}{4}$.

TABLE 4.—*Composite Cardiac Weights* *

	Variate							
					Total			
	LV+IVS		LV+IVS+RV		Muscle Mass		Heart Weight	
	M	F	M	F	M	F	M	F
No emaciation								
1. "Normal"								
n	26	19	21	14	21	14	16	13
Low	122	91	164	126	207	153	256	198
High	203	141	270	179	319	217	390	279
Range ratio	1.7	1.5	1.6	1.4	1.5	1.4	1.5	1.4
Mean	155	115	202	149	254	186	328	244
Median	156	114	193	147	252	190	328	234
Sex ratio	1.37		1.31		1.32		1.40	
2. Old age								
n							4 ‡ †	
Low							345	
High							380	
Median							356	
3. Right ventric. hypertrophy								
n			5 ‡	4 ‡	5 ‡	4 ‡	4 ‡	3 ‡
Low			179	142	219	172	271	198
High			320	218	392	266	468	326
Median			237	160	286	203	334	264
4. Excessive epicardial fat								
n				1 §		1 §	2 §	3 ‡
Low				145		193	373 ‡	304
High							412	393 ‡
Median								336
5. Total								
n	26	19	26	19	26	19	26	19
Low	122	71	164	126	207	153	256	198
High	203	141	320	218	392	266	468	393
Range ratio	1.7	1.5	2.0	1.7	1.9	1.7	1.8	2.0
Emaciation								
n	6	8	6	8	6	8	6	8
Low	113	71	153	85	186	115	207	125
High	152	114	217	144	277	180	316	227
Median	136	97	180	125	214	156	292	197

* In grams.

† Age groups are indicated as follows: ‡ 81-92, § 46-67, ‖ 25-66, ‡ 25-49, § 56, ‖ 56-64, ‡ 56-66.

‡ Had right ventricular hypertrophy as well as excessive epicardial fat. Because the latter affected the total heart weight more decisively than the former the case is listed in Subdivision 4 rather than in Subdivision 3.

TABLE 5.—*Thickness Measurements* *

	L. Ventr.		R. Ventr.		I. V. Septum	
	M	F	M	F	M	F
No emaciation						
1. "Normal"						
n	21	17	11	14	20	15
Low	12	10	3	3	9	11
High	19	18	7	5	20	19
Range ratio	1.6	1.8	2.3	1.7	1.7	1.7
Mean	15	14	4	4	16	14
Median	15	14	4	4	17	14
Sex ratio	1.07		1.0		1.21	
2. Old age						
n			7(1) †			
Low			4			
High			9			
Median			5			
3. R. V. hypertrophy						
n			3(3)	2(3)		
Low			3	7		
High			8	13		
Median			5	—		
4. Total						
n	21	17	21	16	20	15
Low	12	10	3	3	9	11
High	19	18	8	13	20	19
Range ratio	1.6	1.8	2.7	4.3	1.7	1.7
Emaciation						
n	6	7	6	7	6	6
Low	9	12	2	3	10	12
High	20	20	9	6	15	17
Median	12	13	5	4	12	14

* In millimeters; measurements not available for all cases.

† Age groups are indicated as follows: (1) 77-92, (2) 46-56, (3) 25-49.

Because of deviations in weights and ratios brought about by body wasting the emaciated cases are always listed as a separate group.

Component Cardiac Weights (Table 3)

Left Ventricle and Interventricular Septum.—The maxima for both LV and IVS were furnished by a young man of exceptionally powerful build.

In 10 of the 45 nonemaciated cases separation of the left ventricle from the interventricular septum was probably faulty. These cases are listed under the subdivision "Possible Cutting Error."

Right Ventricle.—The highest value among the "normal" cases was provided by the same man who had also furnished the highest weights for LV and IVS.

Among 35 nonemaciated cases without RV overload, the weight of the right ventricle tended to increase with old age in men.

There were 10 cases with unduly heavy right ventricles. An acceptable substrate of RV overload was identified in 9 of the 10 as follows: Bullous emphysema (all in men), recurrent thromboembolism, postradiation fibrosis of the lungs, thoracic kyphosis, and pulmonary infiltration by malignant lymphoma. In no case did RV hypertrophy demonstrably affect the weight of either LV or IVS.

The 10th case was a woman without pulmonary pathology. With 192 gm. of dissectable epicardial fat, or nearly four times the median of the (female) group, the heaviness of RV was interpreted as pseudohypertrophy from excessive interstitial obesity of its wall. There were two other cases (1 man, 1 woman) with excessive epicardial fat that may have had pseudohypertrophy of the right ventricle; although RV was within the normal weight range, the ratio (LV+IVS)/RV was at the lower limits of normal.

Atria.—The weight of the atria tended to be high among men of advanced age. In fact, every one of the eight men aged 75 and over had atrial weights above the median of the men below age 74.

Epicardial Fat.—The weight of the epicardial fat among 22 men below age 77 did not exceed 79 gm., except in 2 persons (9%). By contrast, above age 80, excess amounts were found in every instance.†

In women, the weight of the epicardial fat did not exceed 72 gm. except in 3 of 19 persons (16%). The median was lower for the women than for the men because the number of young persons (below age 40) was greater among the former (6:16) than among the latter (1:20). Whether epicardial obesity in women would have been as characteristic of senescence as it was in men could not be determined as none of the women lived beyond age 68.

† There were only four such cases in the "old age" group, as against seven cases under "right ventricle" and eight under "atria." The reason for this seeming discrepancy relates to the fact that the augmenting effect which senescence had upon these three variates became manifest at different age levels and thus comprised a varying number of persons.

TABLE 6.—*Ratios Between Component and Composite Cardiac Weights*

No. Emaciation	LV+IVS / RV		LV / RV		LV / IVS		IVS / RV		Atria / LV+IVS+RV		Atria / LV+IVS		Atria / RV		Epic. Fat / TMM	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1. "Normal"																
n	14	14	10	10	20	15	10	10	13	14	17	18	13	14	19	15
Low	3.0	3.0	1.7	1.8	1.2	1.2	1.2	1.3	0.18	0.18	0.24	0.23	0.7	0.8	0.13	0.12
High	3.8	3.9	2.2	2.3	1.7	1.7	1.6	1.6	0.32	0.37	0.41	0.48	1.6	1.6	0.37	0.40
Mean	3.4	3.5	2.0	2.0	1.5	1.4	1.4	1.5	0.24	0.25	0.34	0.33	1.1	1.1	0.24	0.27
Median	3.4	3.6	2.0	2.0	1.5	1.3	1.4	1.4	0.24	0.23	0.32	0.32	1.0	1.1	0.25	0.32
2. Old age																
n	7 [1,2]		6 [1]				6 [1]		8 [2]		8 [2]		8 [2]		4 [2]	
Low	2.6		1.6				1.0		0.24		0.32		1.0		0.34	
High	3.4		2.0				1.5		0.34		0.46		1.4		0.48	
Median	2.9		1.8				1.2		0.30		0.40		1.2		0.38	
3. Right ventric. hypertrophy																
n	5	4	4	4			4	4	5	4	1		5	4	1	1
Low	1.3	1.4	0.7	0.8			0.5	0.6	0.21	0.19	0.40		0.5	0.5	0.17	0.19
High	3.1	2.7	1.8	1.5			1.2	1.2	0.26	0.28	--		1.1	1.0	--	--
Medium	2.2	2.4	1.4	1.2			1.0	1.0	0.22	0.25	--		0.8	0.8		
4. Excessive epicardial fat																
n		1		1				1		1		1		1	2 [4]	3 [4]
Low		2.5		1.5				1.0		0.33		0.46		1.2	0.31	0.48
High		--		--				--		--		--		--	0.55	0.99
5. Possible cutting error																
n			6	4	6	4	6	4								
Low			1.4	1.8	1.1	1.1	0.7	1.0								
High			2.5	2.8	2.0	2.6	1.6	1.6								
6. Total																
n	26	19	26	19	26	19	26	19	26	19	26	19	26	19	26	19
Low	1.3	1.4	0.7	0.8	1.1	1.1	0.5	0.6	0.18	0.18	0.24	0.23	0.5	0.5	0.13	0.12
High	3.8	3.9	2.5	2.8	2.0	2.6	1.6	1.6	0.34	0.37	0.46	0.48	1.6	1.6	0.55	0.99
Emaciation																
n	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8
Low	2.3	3.1	1.2	1.6	1.2	1.0	1.0	1.4	0.18	0.16	0.24	0.17	0.7	0.8	0.06	0.03
High	3.7	5.1	2.1	2.9	2.2	1.9	1.6	2.5	0.33	0.36	0.51	0.48	1.5	2.1	0.44	0.32
Median	3.2	4.0	1.9	2.5	1.5	1.3	1.2	1.6	0.23	0.24	0.30	0.30	0.8	1.2	0.16	0.16

* Age groups are indicated as follows: [1] 77-92; [2] 75-92, [3] 81-92, [4] 56-64, [5] 56-66.

Composite Cardiac Weights (Table 4)

Combined Ventricles and Total Muscle Mass.—In only two of the nine cases with RV hypertrophy was the weight increase of the right ventricle of sufficient magnitude to raise total ventricular mass and TMM appreciably.

Total Heart Weight.—THW tended to be high in association with advanced age,‡ epicardial obesity and RV hypertrophy.§

Thickness Measurements (Table 5)

An appreciable difference between men and women was encountered for the thickness of IVS but not for that of LV or RV. The high of 7 mm. for the "normal" right ventricle was furnished by the same man who also contributed the highest weights for the ventricular components.

As in the case of the weights, the thickness of RV was comparatively high among aged men (77 years and over) and among cases with RV hypertrophy and RV pseudohypertrophy.

Ratios

Weight Ratios (Table 6).—As expected, advanced age (in men), epicardial obesity, and RV overload influenced any ratio entered into by RV or atria.

Sex Ratios (Tables 3-5).—By sex ratio was meant the male:female quotient of the median of the various measurements. All variates listed in Tables 3 and 4 were from 19% to 48% greater in men than in women (sex ratios, 1.19-1.48).

The sex ratios for wall thickness of the various components (Table 5) were lower than those for the weights.

Range Ratios (Tables 3-5).—By range ratio was meant the quotient between the highest and lowest values for each variate among the members of the same sex. As regards the "normal" cases, the range ratios were reasonably similar for the partitioned and composite cardiac weights with the exception of atria and epicardial fat.

Inclusion of the cases with RV hypertrophy raised the range ratio for RV appreciably. While in "normal" persons the heaviest RV weighed 80% more than the lightest, the difference amounted to as much as 290% and 240% for men and women, respectively, when the cases with RV hypertrophy were included.

Emaciation

Fourteen persons were emaciated. All but three displayed at least one component heart weight which fell outside the range of the nonemaciated "normal" cases. Next to the epicardial fat, the variate most frequently below "normal" was RV (in 6 of the 14 cases). Among the remaining eight cases, RV was within the "normal" range in the absence of RV overload or was above it when RV overload was present. Among the women, the medians of nearly all the component and composite weights roughly equalled the lowest value of the nonemaciated group.

The ratios which most frequently deviated from "normal" were (LV+IVS)/RV and LV/RV, which were higher than "normal" unless there was RV overload, and epicardial fat/TMM, which was lower than "normal."

Congestive Heart Failure

According to the clinical records, 5 of the 59 cases (8%) were in congestive heart failure. None of the five (3 men, 2 women) was emaciated. With one exception, failure was right-sided. There was right ventricular hypertrophy (cor pulmonale) in three. The

‡ In no case of old age was the increase of RV and atria large enough to raise total ventricular mass or TMM appreciably. This explains why in Table 4 no entries are recorded for "old age" under these particular variates. By contrast, the increase of epicardial fat was of a magnitude sufficient to affect THW.

§ Of the nine cases listed under "right ventricular hypertrophy," there were two (one man, one woman) whose hearts exhibited unduly large amounts of epicardial fat. Because their total heart weights were augmented relatively more by the latter than by the former, these two cases are recorded under "excessive epicardial fat."

TABLE 7.—*Correlations Between Cardiac Muscle Weights* *

Pairs of Variates		Cases	Men			Women		
			n	*r*	*P*	*n*	*r*	*P*
LV+IVS	RV	No RVH	21	0.83	0.01	14	0.76	0.01
LV	RV	No RVH	21	0.68	0.01	14	0.60	0.05
Atria	RV	No RVH or old age	No correlation			14	0.54	0.05
LV+IVS	Atria	No old age	17	0.59	0.02	18	0.34	>0.10
LV+IVS+RV	Atria	No old age	17	0.53	0.05	18	0.43	0.10
TMM	THW	All	25	0.92	0.01	18	0.61	0.01

* *n* indicates number of cases; *r*, correlation coefficient (for perfect correlation, *r*=1); *P*, probability that there is actually no correlation in the population of which this is a sample (*P*≦0.05 means that the correlation coefficient is "significant"); RVH, right ventricular hypertrophy; no correlation, *r*<0.33 (see text); TMM, total muscle mass; THW, total heart weight; All, all nonemaciated cases except those with severe right ventricular hypertrophy (RV>LV).

fourth case was a woman who, in spite of bullous emphysema and bronchial asthma of five years' duration, failed to exhibit right ventricular hypertrophy. In the fifth case, an 83-year-old man, no adequate anatomic reason for cardiac failure could be found. The weights and ratios were all within the "normal" range for his age; there was neither pulmonary pathology nor myocardial or valvular disease, and the coronary arteries showed merely a few points of 1+ narrowing. The case may have been an example of senile heart failure postulated as an entity by Dock.[16]

Correlations ‖

Correlations Between Cardiac Muscle Weights (Table 7).—Statistically significant correlations were noted in both sexes between LV and RV and between (LV+IVS) and RV. These correlations were masked in the presence of RV hypertrophy. Lesser correlations existed between atria and the component and composite weights of the ventricles.

Correlations with Epicardial Fat (Table 8).

In both sexes, the epicardial fat was significantly correlated with the weight of the atria but not with total or ventricular muscle mass.

No correlation was demonstrated between epicardial fat and panniculus adiposus. In emaciation, however, there was usually a concomitant reduction of both.

‖ All correlations were based on nonemaciated cases.

TABLE 8.—*Correlations with Epicardial Fat* *

Second Variate	Cases	Men			Women		
		n	*r*	*P*	*n*	*r*	*P*
LV+IVS	All	No correlation			No correlation		
RV	No RVH	No correlation			No correlation		
Atria	All	25	0.45	0.02	18	0.48	0.05
TMM	All	No correlation			No correlation		
THW	All	25	0.59	0.01	18	0.92	0.01
Panniculus adiposus	All	No correlation			16	0.34	>0.10

* For explanations see Table 7.

Correlations with Age (Table 9).—For neither sex was any correlation established between age, on the one hand, and TMM, (LV+IVS) or RV, on the other. Total heart weight did increase substantially with age in women though not in men. The weight of the combined atria was correlated with age in both sexes.

TABLE 9.—*Correlations with Age* *

Second Variate	Cases	Men			Women		
		n	*r*	*P*	*n*	*r*	*P*
THW	All	No correlation			18	0.42	0.10
TMM	All	No correlation			No correlation		
LV+IVS	All	No correlation			No correlation		
RV	No RVH	No correlation			No correlation		
Atria	(*a*) All	25	0.49	0.05	18	0.45	0.10
	(*b*) Limited body height †	14	0.73	0.01	9	0.61	>0.10
Epicardial fat	All	25	0.47	0.02	18	0.47	0.05
Panniculus adiposus	All	No correlation			16	0.51	0.05

* For explanations see Table 7.
† Men, 150-169 cm.; women, 162-175 cm.

There was significant correlation between age and epicardial fat, among both men and women. This correlation was not biased by the inclusion of cases with undue epicardial obesity. In fact, the correlation became stronger if these subjects were omitted.

There was significant correlation between age and abdominal panniculus adiposus among women. Among men, leanness (panniculus adiposus below 2 cm.) occurred at all ages. Among women, leanness was confined to young persons 36 years of age and under. Conversely, obesity (panniculus adiposus, 3 cm. or above) was conspicuously less common among men than among women.

TABLE 10.—*Correlations with Body Height* *

Second Variate	Cases	Men			Women		
		n	*r*	*P*	*n*	*r*	*P*
THW	All	No correlation			No correlation		
TMM	All	No correlation			17	0.47	0.05
LV+IVS	All	No correlation			17	0.39	>0.10
RV	No RVH	No correlation			No correlation		
Atria	(*a*) No old age	17	0.53	0.05	17	0.38	>0.10
	(*b*) Limited age †	13	0.68	0.02	9	0.70	0.05
Epicardial fat	All	No correlation			No correlation		
Panniculus adiposus	All	No correlation			16—0.34		>0.10

* For explanations see Table 7.
† Men, 56-74; women, 21-49.

Correlations with Body Height (Table 10).

In men, body height was not significantly correlated with any of the component or composite cardiac weights except the atria. Among the women, body height was significantly correlated with total muscle mass. In both sexes the correlation of atria with body height became greater by limiting the age range under consideration, i. e., by reducing the independent effect of age upon atrial weight (see above).

Correlation Between Thickness and Muscle Weights.—No correlation could be established between wall thickness and weight of LV, IVS or RV.

Comment

A. General.—Although elaborate, the procedure here employed was not altogether free of objections. In the first place, what has been presented as muscular weights included bits of epicardial fat impossible to remove by sharp dissection, especially about the posterior aspect of the interatrial septum. The error was considered negligible except, perhaps, for the atria. The muscle weight was also modified by nondissectible interstitial fat, especially in the right ventricle. The weight of injection mass remaining in intramyocardial arteries was irrevelant, an estimated 2-3 gm. for the entire heart.

Secondly, the technique of isolating the free walls of the chambers from their septal insertions was not a precise undertaking. It seemed reliable enough for the right ventricle, whose borders with the interventricular septum were distinctive. It was difficult with respect to the atria, which, for this reason, were treated as a unit consisting of the free walls and interauricular septum. The technique was of uncertain exactitude as regards the separation of the free left ventricle, a shortcoming also remarked on by previous workers and not circumvented by our preliminary "unrolling" of the cardiac cone. For this reason, the weight of the free left ventricle was accepted only if the ratios LV/RV and (LV+IVS)/RV were compatible with each other. If not, the cases were listed as "cutting errors." Previous workers had turned to expressing the weight of the left ventricle as the sum of its free portion plus a fraction of the interventricular septum apportioned either by computation based on the relative weights of the free left and free right ventricles [5,17] or by arbitrary anatomic division through the septum along the line of junction of the scroll muscles (the "white line" of Herman and Wilson [18]). Either procedure, it seemed, merely substituted one uncertainty with another.

Thirdly, a weight loss of about 5% from evaporation was unavoidable in the course

of injection and dissection. The partitioned weights were not corrected for this loss but instead were reported as found. For the same reason, all composite weights including total muscle mass and total heart weight were arrived at by computation.

Because all the specimens were processed identically, the values obtained were deemed comparable and, at any rate, free of bias.

Evaluation of abnormal work demands on left and right ventricles as here practiced is admittedly contestable. The criteria adopted and some of the reasons have been given under "terminology." Additional comments seem appropriate.

Fully cognizant of the fallibility of blood pressure records on grounds of technique, emotional state, disease, etc. and aware of the variability of blood pressure with bed rest, medication, etc., we chose for classification the reading recorded upon admission to the hospital unless such was clearly and grossly unrepresentative of preceding or subsequent observations. Instead of selecting from recommendations offered by others, we deduced the upper limits of "normal" blood pressure from the distribution curve of the left ventricular weights among our own cases choosing those values which would not exclude any seemingly "normal" hearts. Because of the rise of blood pressure with age, our upper limit of 170/90 mm. Hg might not have been found had the analysis been based predominantly upon young persons. In fact, three cases in this very series with blood pressures below 170/90 ¶ had left ventricular weights much in excess of our "normal" range. In these persons who were excluded from the present report, a blood pressure of less than 170/90 was physiologically equivalent to left ventricular overload and, in fact, had so been diagnosed clinically. Conversely, we had only one case with blood pressure above 170/90 and none above 170/100 that did not display at least some measure of left ventricular hypertrophy. There were six cases # with systolic blood pressures of between 140 and 170 and with diastolic pressures no higher than 90 whose heart weights fell within "normal" limits. Moreover, in none of these cases had a clinical diagnosis of hypertension been made.** These cases were included in this current report.

Even more difficult than the evaluation of systemic blood pressure was the estimate of workload in the lesser circulation. In no case did we have in vivo measurements of pulmonary arterial pressure. Therefore, any increase of vascular resistance had to be deduced from the distribution curve of the weights of the right ventricles and from the distribution of the ratios LV/RV and (LV+IVS)/RV. By correlating the weights of the right ventricle with clinical, roentgenological, and postmortem findings, the analysis yielded information on which pulmonary conditions did and which ones did not tend to raise ventricular workload appreciably. Right ventricular hypertrophy was found to be associated with bullous emphysema (four out of five cases), recurrent thromboemboli (two of two cases), and postradiation fibrosis, lymphomatous infiltration of the lungs, and thoracic kyphosis (one case each). There was no hypertrophy of the right ventricle with senile emphysema, fresh thromboemboli, or pulmonary atherosclerosis. Worthy of note was the absence of measurable right ventricular hypertrophy (*a*) in a case of bronchial asthma and bullous emphysema and (*b*) in three cases of carcinomatous metastases to the lungs including a case of carcinoid of the jejunum. Of course, a right ventricle falling within "normal" weight limits does not rule out hypertrophy but may signify merely that pulmonary hypertension was of a degree insufficient to increase the weight of the right ventricle beyond the limits established.

¶ Man, age 29, B. P. 145/90; woman, age 31, B. P. 160/90; woman, age 66, B. P. 170/80.

Three men, aged 48-72, and three women, aged 45-55.

** The New York Heart Association[19] defines 140/90 as the upper limit of normal but adds that "hypertension is not to be considered . . . when the systolic phase alone is elevated" (p. 17).

B. Statistical Considerations.—Without bias on whether normality is a scientific fact or a philosophical concept, the weight ranges for "normal" heart were arrived at by a process of exclusion. We accepted as "normal" any heart which was not manifestly abnormal being free of myocardial pathology and of increased work load upon the left ventricles.

The emphasis on the left ventricle as the chamber of reference was dictated by clinical considerations related to the overriding importance of systemic hypertension. By our definition, cases had to be included in this "normal" series which though free of left ventricular overload had, nonetheless, been subjected to increased work demands on their *right* ventricles. These cases are identified and, whenever indicated, treated separately. In none of these cases did the weight of the left ventricle fall outside the weight limits of those "normal" cases which were not exposed to right ventricular overload.

Although we can not claim comprehensive representation for the small size of our "normal" sample, we do believe that the weights and correlations here recorded constitute reasonable approximations of what might have been found had the sample been larger. This belief is supported by the similarity of our data with those of several other studies which like ours were based on partitioning techniques.[4,17,20] Moreover, this "normal" sample is thought to furnish an adequate basis for the evaluation of various influences acting upon the component cardiac weights under conditions both physiological (body height, obesity-leanness, age) and pathological (coronary artery disease, hypertension, etc.).

By combining a partitioning technique with appropriate statistical procedures, the disadvantage inherent in the small size of our sample, we believe, was effectively overcome. Moreover, certain interrelations could be clarified which remained obscure or contradictory in other studies based on total heart weight, however large the samples.

In the interpretation of the data we have tried to follow Snedecor's [21] admonition not to get lost in "minute details of sample variation neglecting the summarization of the data" nor to rush "carelessly toward averages and other generalizations." Rather, we have attempted "to distinguish normal variations from aberrant observations," to "judge . . . summaries and . . . population inferences" and to draw "conclusions against this vivid background of sample facts." We have interpreted our values in terms of ranges and quartiles in order to show the actual distribution as we found them to occur. We have thereby disregarded the standard deviation of the mean as customarily recorded for the calculation of the fiducial interval, i. e., the probable range of normal values. Such a range of normality based on $m \pm 2\sigma$ would have included values for (LV+IVS) which were observed in reality only in emaciated persons and excluded others which were actually observed.

There is unanimity in the literature [1,3-5] that, everything being equal, the hearts of men are heavier than those of women. Our data are in full accord with this and indicate that this sex difference pertains to the cardiac components as well. Indeed, the sex ratios (median ♂ : median ♀) of component and composite cardiac weights are so similar as to suggest that this weight differential may be of the nature of a biological constant.

C. Correlations.—For the purpose of establishing "normal" correlations, the emaciated cases were omitted altogether, as were two cases with hypertrophied right ventricles, so severe as to exceed the weights of their left ventricles. A selection was also made among the other cases whenever a given correlation might have become modified because of cutting errors, excessive epicardial fat, old age, or right ventricular hypertrophy.

Emaciation exerted a profound influence upon the cardiac weight. Emaciation, it seemed, was not just obesity with a minus sign. Our data suggested that the slow gain of, say a 140 lb. person to 180 lb. affected

the heart only little if at all and then mainly in the epicardial fat fraction. Contrariwise, the rapid loss of 40 lb., from 180 lb. to 140 lb., incident to wasting disease reduced the heart weight substantially in both the muscle and the epicardial fat fractions, though more so in the latter.

In the correlation studies we have placed the emphasis on the size of a correlation coefficient rather than on its statistical significance, on the reasoning that there is more meaning to a coefficient of 0.50 which, because of the smallness of the sample, falls short of statistical significance than to an r of 0.25 which, because of the largeness of the sample, is statistically significant.††

The closer r is to unity, the more perfect is the correlation. While correlations of 0.99 are not unheard of in biological analyses, smaller ones are commoner. Thus, the correlation coefficient between such closely interdependent variates as stature and span [22] was only 0.60-0.83, i. e., the same order of magnitude as we found for the muscular components of the cardiac ventricles.

Coefficients of about 0.50 may indicate opposing correlations or else combinations with noncorrelating factors. Thus, the correlations which the atria displayed with both age and body height became stronger with either when the effect of the other was excluded from calculation.

Whether statistically significant or not, correlations below 0.40 call for a breakdown of the group for identification of opposing influences.

A correlation coefficient of 0.33 or less was interpreted as "no correlation," first, because in our small sample such correlations were not significant ($P>0.10$), and second, because no opposing influences could be demonstrated.

1. Correlations Between Cardiac Muscle Weights (Table 7): The various muscular components of the "normal" heart were significantly correlated with each other in both sexes. This is as one would have expected of an integrated viscus. As compared with the men, the lack of significant correlation between atria and (LV+IVS) in women ($P>0.10$) was ascribed to an insufficient number of cases, as was the generally higher P (less significance) of other correlations.

2. Correlations with Epicardial Fat (Table 8): In neither sex was there any correlation between the muscle mass of the combined ventricles and the weight of the epicardial fat, an indication that the latter did not participate in the organ integration which characterized the muscular components. This lack of correlation furnished additional justification, if such had been needed, for considering epicardial fat separate from muscle mass. Because of the supposition that interstitial obesity of the right ventricle increased with a rise in dissectible epicardial fat, it was worthy of note that the weight of the free right ventricle did not correlate with the weight of the (total) dissectible epicardial fat.

There was in both sexes a significant correlation between the weight of the atria and the weight of the epicardial fat. This correlation may be explained either on grounds of difficulties encountered in the quantitative removal of the epicardial fat from the atria or on grounds of the independent association of epicardial fat and atria with age and hence with each other.

Excluding emaciated states, the thickness of the abdominal panniculus adiposus and, by inference, the status of obesity-leanness of the body showed no correlation with the amount of dissectible epicardial fat in either sex. Although in a previous communication [13] we did report significant correlations in both sexes, the contradiction is more apparent than real, because no distinction had then been made between atrophic, normotrophic, and hypertrophic hearts or between emaciated and nonemaciated persons.

Women tended to have proportionately the larger amounts of dissectible epicardial fat, inasmuch as age for age the weight of

†† The smaller the sample the larger must be the correlation coefficient. For instance, for a sample of 25, significance lies with $r \geqslant 0.40$; for a sample of 13, r must be $\geqslant 0.55$.

the epicardial fat was about the same in men and women though the total muscle mass was about 25% less among the latter. This partially accounted for the fact (*a*) that the correlation between total heart weight and total muscle mass was much greater in men (r=0.92) than in women (r=0.61) and (*b*) that the opposite was true for the correlation between total heart weight and epicardial fat.

3. Correlations with Age and Body Height (Tables 9 and 10): Relative to the effects of body height and age upon the component cardiac weights, scatter diagrams indicated group differences in the sense that low values tended to be associated both with short stature and younger ages while high values tended to be associated with both tallness and older ages. By expressing this tendency at grouping in terms of correlations we found not only statistical significance but also avoided what others have erroneously implied, namely, that there exists a *linear* regression of cardiac weight upon body height [1] and possibly upon age.[2]

Epicardial fat was correlated with age in both sexes excluding emaciated persons. This, too, was at variance with our previous report [13] and probably again for the reason that in the former series the cases were totally unselected. The inference is clear: Obesity-leanness of the body, epicardial fat, and age were interrelated differently among emaciated and nonemaciated persons, among men and women, and among normotrophic and hypertrophic hearts (i. e., among normotensive and hypertensive persons).

The weight of the atria was correlated with age in both men and women. In view of the correlation between atria and epicardial fat, the increase of atrial weight with advancing age may denote an age-conditioned increase of (nondissectible) atrial fat rather than an increase of atrial muscle mass.

4. Correlations with Abdominal Panniculus Adiposus. No correlation existed between the thickness of the panniculus adiposus and either total heart weight or total muscle mass (no table). In view of the unanimity of opinion in the literature that body weight—the composite of musculoskeletal development plus obesity-leanness—and total heart weight are correlated with each other, we infer that either the abdominal panniculus adiposus is not a good reflection of body weight or else that total heart weight is a function of somatotype and musculoskeletal development rather than of obesity-leanness.

To our knowledge, these constitutional aspects of body weight have not, heretofore, been correlated independently with total and fractional heart weights. Zeek [1] has mentioned the element of body build by observing that persons (12 out of 523 males) with "unusually powerful muscular development" had heavier hearts than the remainder of the subjects categorized as either "emaciated," "normally nourished," or "obese." This observation is in keeping with our own experience. For instance, in our series the maxima for the partitioned ventricular weights were all furnished by a young man of particularly powerful build. A correlative study is needed based on anthropomorphic criteria and on the specific gravity of the body rather than on either crude body weights or on the thickness of the abdominal panniculus adiposus. With Roessle and Roulet [4] we suspect that musculoskeletal development may influence myocardial mass to as great an extent as any of the parameters here analyzed. We also suspect that any correlations which did exist with body height and body weight were at least partial expressions of body constitution.

Summary and Conclusions

Based on the method of Müller, the hearts of 292 adults were first freed of their epicardial fat. The heart muscle was then partitioned so as to allow for separate weight determinations of the free portions of the right and left ventricles, the interventricular septum, and the atria. From these "component" cardiac weights various combinations, i. e., "composite" cardiac weights, were calculated.

The current report deals with 59 of these cases selected on the basis of being free of myocardial pathology, valvular disease, left ventricular overload, and severe coronary arteriosclerosis. The "normal" values for the component and composite cardiac. weights were determined by omitting—wherever pertinent—those of the 59 cases subjected to the modifying factors of emaciation, right ventricular overload, advanced age, and excessive epicardial fat. These "normal" values are tabulated in terms of ranges, medians, and means to serve as the base line for subsequent comparison with pathological hearts.

In both men and women the maximum of the ventricular components and of the composite cardiac weights was approximately 50% greater than the minimum. Each of these muscular variates was approximately one-third heavier in men than in women. The thickness of the left ventricle and interventricular septum was also greater in men than in women.

The analysis of the data included correlations of the various component and composite cardiac weights with each other as well as with extracardiac factors which might conceivably influence these weights.

The component and composite ventricular muscle weights were significantly correlated with each other.

There was no correlation between thickness and weight of the ventricular components.

There was no correlation between epicardial fat and the total or ventricular muscle masses, indicating that its quantity depended on factors other than those governing the weight of the myocardium.

Obesity-leanness of the body (as inferred from the thickness of the abdominal panniculus adiposus) was not correlated with the quantity of dissectible epicardial fat.

Age did not influence the weight of the myocardium. However, there was a significant correlation between age and dissectible epicardial fat. The weight increase of the atria with age and also the tendency of the right ventricle to be somewhat heavier in the old were probably related to a concomitant increase of nondissectible epicardial fat.

No correlation could be established between total heart weight and either body height or obesity-leanness. However, there was significant correlation between body height and total cardiac muscle mass in women though not in men. The opinion is expressed that factors of body constitution other than those here explored may substantially influence the component and composite cardiac weights.

Emaciation was found to constitute a potent modifying influence upon cardiac weights, especially upon the epicardial fat and the right ventricle.

Correlations of cardiac weights with each other and with extracardiac variables may be unrevealing or indeed deceptive unless the cases under consideration are identified and treated separately according to the presence or absence of cardiac hypertrophy or cardiac atrophy.

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Cancerous Mixed Mesodermal Tumor of the Ovary

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Mixed mesodermal tumors of the uterus and adnexa, most of them cancerous, have a uniquely specific histologic structure and commonly are regarded as a rare form of tumor growth. The uniqueness of their histologic structure rests upon the presence of several varieties of atypical mesoblastic and epithelial tissues, such as undifferentiated mesodermal cells, immature cartilage or osteoid tissues, rhabdomyosarcoma cells, and atypical epithelium, clearly arranged in tubules or papillae and less specifically in mosaics. Mixed tumors of the corpus of the uterus are much more frequent than those of the cervix, and those of uterine appendages are rarest. Wurtz,[1] in 1949, among 18 of these mixed tumors found in surgical tissues at St. Luke's Hospital, reported 14 in the corpus, 2 in the cervix, and 1 each in the vagina and in the left broad ligament. Glass and Goldsmith[2] tabulated 58 tumors in the body and 36 in the cervix; Sternberg and associates,[3] 15 of 21 tumors in the corpus, 3 in the cervix, and 3 in the vagina, and Taylor,[4] 26 of 40 tumors in the corpus, 11 in the cervix, and 3 in the vagina.

According to Perlstein,[5] the first mixed tumor of the uterus was reported in 1854 by Wagner[6] as a chondrosarcoma. Since then many of these tumors have been recorded, on the basis of their histologic structure, as combinations of carcinoma and sarcoma tissues, such as carcinochondromyxofibrorhabdomyo- or leiomyosarcoma. This unsatisfactory terminology does not express the specific histologic structures in a tumor, because every mixed tumor does not have all of the tissue elements mentioned, and many have undifferentiated cells that cannot be recognized as derivatives of any specific mesoblastic tissue. Wilms[7,22] clarified much of the confusion in the terminology of these tumors in his monograph, published in 1899, on urogenital tumors and stated that "displaced rests" of primitive mesodermal tissue could remain dormant and give rise in later years to a mixed tumor. Kehrer[8] in 1906 proposed that the term mixed mesodermal tumor would indicate satisfactorily their complex embryonic mesodermal tissue structure and would express a relationship to the possible origin of these tumors from embryonal mesodermal residues. Meikle[9] agreed with this terminology because the name would include those tumors with glands and epithelial tissues, comparable to structures in the endometrium, which he considered to be mesodermal in origin. Duggan[10] disagreed with this opinion because he did not accept fully the mesodermal origin of glandular epithelium in the endometrium. He considered the term mixed tumor more acceptable and sufficiently descriptive. Long usage of the name proposed by Kehrer, however, has established rather firmly the term mixed mesodermal tumor for these neoplasms of the female genital tract containing the heterologous atypical tissues mentioned.

Similar tumors of the Fallopian tubes have been designated as spindle-cell sarcoma, round-cell sarcoma, myxosarcoma carcinosarcoma, mesothelioma, celomic mesothelioma, and periendothelioma, and the terms rhabdomyoma, rhabdomyosarcoma, and Müllerian carcinosarcoma have been applied to the same growths of the ovary. To date, no description of such a mixed tumor of the uterine appendages has been published under the title of mixed mesodermal tumor.

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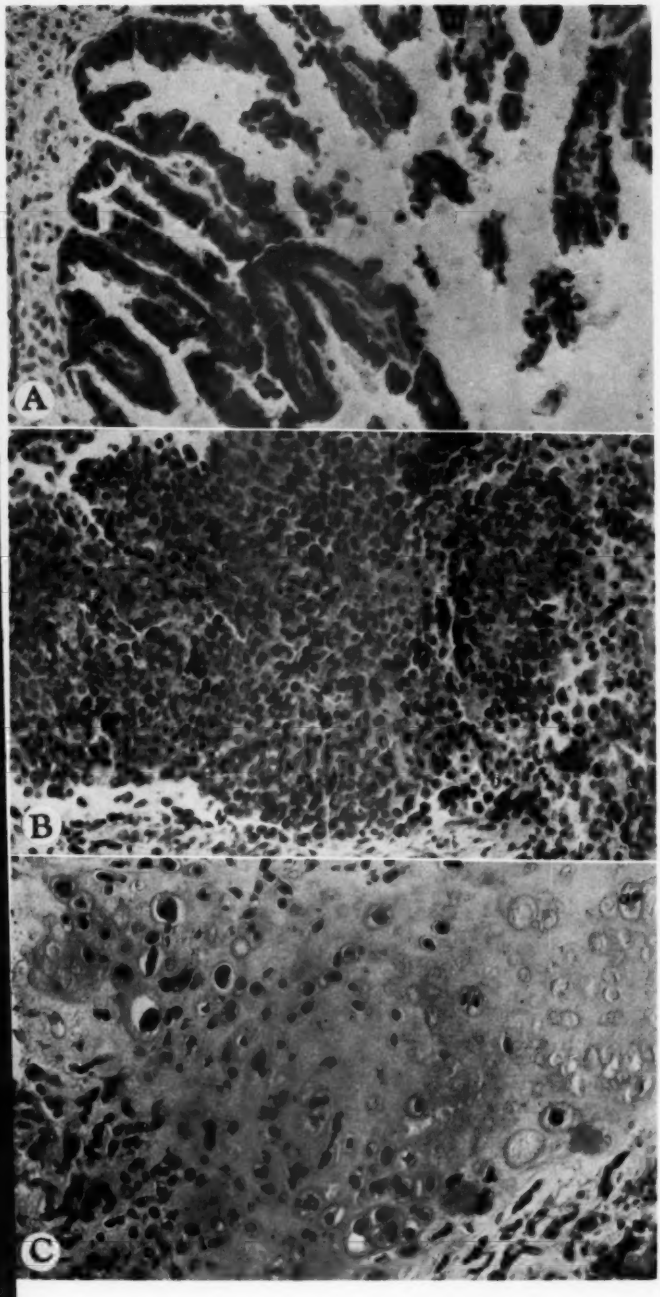

Fig. 1.—Photomicrographs of the ovarian tumor tissues illustrating (*A*) papillary carcinoma, (*B*) masses of atypical epithelial cells in mosaics, and (*C*) a mass of atypical hyaline cartilage.

Report of a Case

During 1957 and 1958 a Negro woman, aged 58, was admitted three times to St. Luke's Hospital as a patient. The first time she had a respiratory infection and promptly recovered. On April 10, 1958, she was admitted to the service of Dr. George C. Finola because of a mass in the lower portion of the abdomen, noticed for a month, associated with uterine bleeding. When examined, the mass in the abdomen was firm, painful, and about the size of a four months' pregnancy. The diagnosis of uterine fibroid was made. The laparotomy, on April 22, disclosed a huge multilocular cystic and solid tumor of the left ovary, adherent to the regional abdominal structures. The capsule of the mass was torn during removal, and hemorrhagic fluids escaped. The left ovarian tumor, the right ovary, both Fallopian tubes, and the uterus were removed. She recovered promptly, but returned on July 8, with diarrhea, then developed abdominal pain, associated with vomiting and bowel obstruction. A transverse colostomy afforded relief for the bowel obstruction, but she died on Aug. 5, 1958.

Surgical Tissues.—The torn, solid and cystic mass removed from the region of the left ovary was 12×10×8 cm. The largest cyst, ruptured, was estimated to have been about 12 cm. in maximum diameter, and many other, secondary cysts were in the solid tissues. The solid portions were soft, gray-red tissues. The left Fallopian tube could be traced for a short distance and had a nodule 2 cm. in diameter in the outer portion of its wall. The solid tissues of the tumor were torn and had been sectioned in many places. The small uterus complete was 8.5 cm. long and 4×2.5 cm. between the horns. The Fallopian tubes and ovaries had been amputated. The right Fallopian tube and ovary, as separate tissues, were together 4×1.5×0.8 cm.

The tissues of the ovarian tumor histologically had a supporting stroma and cellular masses of atypical epithelial cells, medium in size, generally arranged in mosaics (Fig. 1*B*) and with a moderate amount of granular cytoplasm and a large vesicular or darkly stained nucleus. Many of these cells were in mitosis. Occasionally there were epithelial papillae, ducts, and papillary cysts (Fig. 1*A*). Scattered in these cellular cancerous tissues were islets of poorly differentiated hyaline cartilage (Fig. 1*C*). Rhabdomyosarcoma cells were not identified in these tissues. The right Fallopian tube and the uterus had only the changes of senile involution. The diagnosis, on the basis of histologic structure, was cancerous mixed mesodermal tumor of the left ovary. The uterus and the right Fallopian tube and ovary had no significant changes except for a small intramural fibromyoma in the body of the uterus and senile involution.

Necropsy.—The essential portions of the anatomic diagnosis of the complete necropsy are as follows: healed surgical panhysterectomy—cancerous mixed mesodermal tumor of the left ovary; recurrent cancerous mixed mesodermal tumor of the pelvis; disseminated cancerous mixed mesodermal tumor metastases of the parietal and visceral peritoneum of the abdomen, and pleura of the left lung; cancerous mixed mesodermal tumor invasion of the rectus muscles of the abdomen; bilateral obstructive hydroureter and hydronephrosis of the kidney; recent surgical colostomy of the transverse colon, etc.

The emaciated body of this Negro woman weighed 130 lb. The midline of the abdomen had an old laparotomy scar between the umbilicus and the symphysis pubis and a recent functional colostomy of the transverse colon to the left of the umbilicus but near the midline. The peritoneal surfaces of the abdomen had many soft gray metastases 2 to 3 cm. in diameter, and several nodules of tumor tissue, 2 by 2.5 cm. in diameter, were in the rectus muscles 3.5 cm. above the symphysis. The loops of small bowel were matted together by fibrous adhesions. A mass of recurrent tumor tissue filled the small pelvis and extended 9 cm. above the symphysis. Loops of small and large bowel were adherent to this mass. Both lungs had hypostatic edema and hyperemia, and the upper lobe of the left lung had a pleural metastasis 1.5 cm. in maximal diameter. The ureters were obstructed by the mass in the pelvis, and both kidneys were hydronephrotic. The upper pole of the left kidney had a cortical adenoma 2 mm. in diameter. No other metastases were found.

The large tumor mass which filled completely the small pelvis consisted of soft gray, solid, and pseudocystic tissues with focal hemorrhages and regions of necrosis.

Histologic examinations of the tissues obtained by necropsy demonstrated a variety of atypical mesodermal and epithelial tissues in the recurrent tumor and in the metastases. The pelvic tumor had undifferentiated mesoblastic cells mingled with scattered islets of immature hyaline cartilage (Fig. 2*A*). The cellular tissues had rhabdomyosarcoma cells of several forms (Fig.

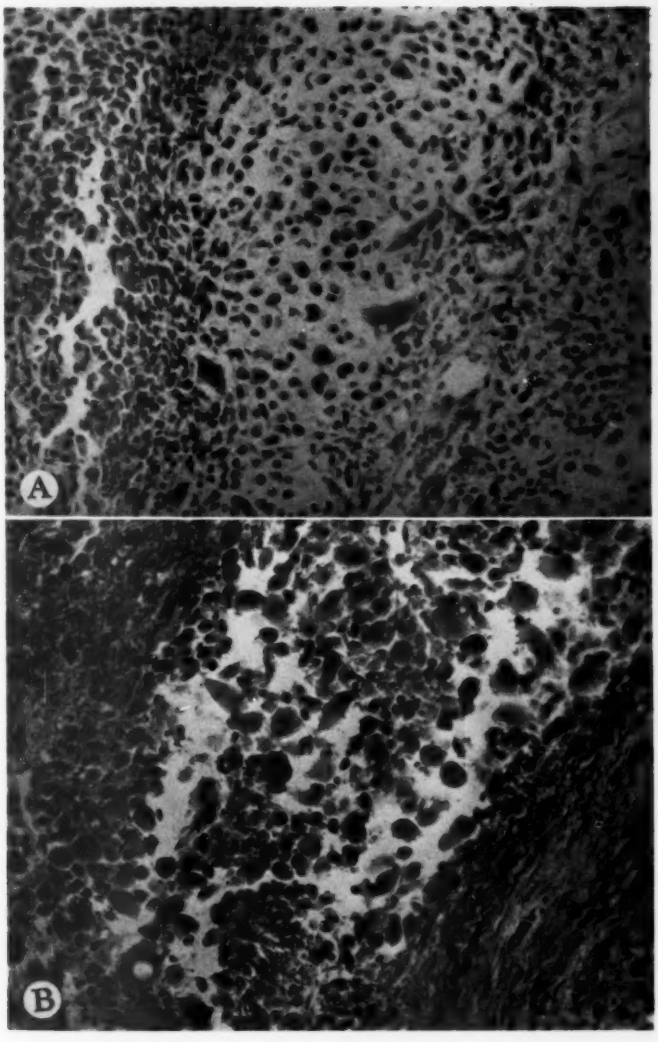

Fig. 2. — Photomicrograph illustrating (*A*) a mass of atypical cartilage and undifferentiated mesoblastic cells in tumor tissues metastatic in an abdominal lymph node, and (*B*) aggregates of large atypical mesoblastic cells, resembling rhabdomyosarcoma, in the recurrent tumor of the pelvis.

2*B*). These were spindle cells, so-called "comet-form" cells, and band cells with a large vesicular nucleus and an acidophilic cytoplasm. The cytoplasmic processes had the cross striations of skeletal muscle fibers (Fig. 3). There were also large rounded cells with concentrically arranged fibrils with coarse granules (Fig. 3*C*). Glands and papillae in the tumor tissues constituted the epithelial components. The metastases in the abdomen were similar, but the pleural metastasis of the left lung had only glandular and papillary epithelial structures.

Comment

Mixed mesodermal tumors of the female genital structures occur at all ages. Vaginal tumors occur in infants and children, those of the cervix during the active menstrual years, and those in the corpus of the uterus usually after the menopause. Scheffey and associates [11] analyzed the reports of 21

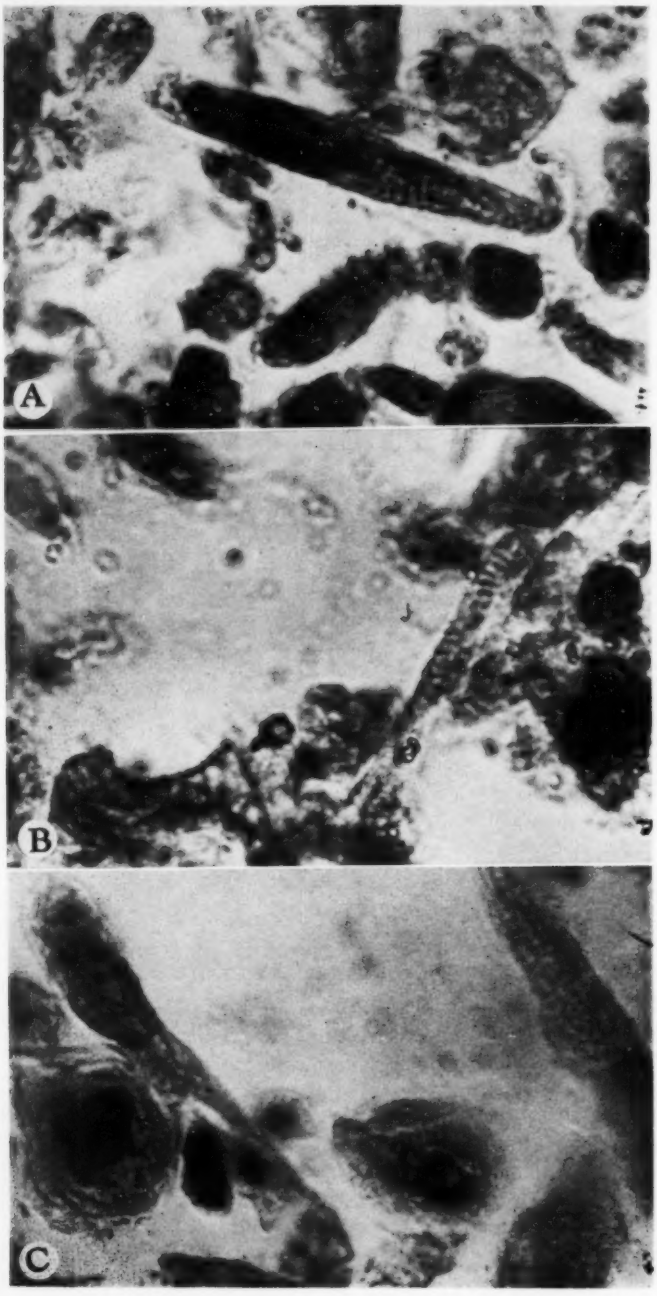

Fig. 3. — Photomicrograph illustrating several rhabdomyosarcoma cells with cross-striated fibers and, in *C*, a round cell with concentrically arranged granular fibers.

sarcomas of the Fallopian tube and noted that the ages of these patients ranged between 40 and 70 years. Sandison,[12] on the basis of his and five other reports of rhabdomyosarcoma of the ovary, stated that the age incidence ranged between 2½ and 60 years. These tumors histologically had only the rhabdomyoblasts and other sarcoma tissues, but not cartilage.

The clinical symptoms of the mixed mesodermal tumor of the uterus and ovary include metrorrhagia, thin or hemorrhagic vaginal fluids, dysuria and hematuria, abdominal distress, and loss of weight. Abdominal pain is the most frequent symptom. Menorrhagia or metrorrhagia occurs in the premenopausal group, and profuse bloody or watery vaginal discharge is a feature of the postmenopausal group. The patient of our report for six months had abdominal pain and distention associated with urinary frequency.

Metastases of mixed mesodermal tumors of the uterus are rare, according to Duncan and Fahmy [13] and Amolsch.[14] Sternberg and associates [3] emphasized the local extension of the tumor from the uterus into the pelvis and the abdomen. Apart from the local invasion, distant metastases have been reported in the lungs, liver, peritoneum, pleura, omentum, and mediastinal lymph nodes.

The microscopic structure of the metastases of mixed mesoblastic tumors have been reported as that of pure sarcoma, papillary adenocarcinoma, or rhabdomyosarcoma. As mentioned before, the histology of mixed mesodermal tumors is characterized by the presence of several heterologous tissues. The tumor has atypical gland-like or papillary structure with columnar epithelium or solid aggregates of undifferentiated epithelial cells and the atypical mesodermal cells. Sternberg [3] stated that mixed mesodermal tumors have the various carcinoma structures which occur in the endometrium, the cervix, the Fallopian tubes, and the vagina. However, the mesoblastic tissues are analogous to those in the Wilms tumor of the kidney, such as mixed mesenchymal sarcoma. The commonest heterotopic tissues are cartilage, adipose tissue, and striated muscle cells. Liebow and Tennant [15] reported cartilage in 42 of 65 tumors. Glass and Goldsmith [2] gave an incidence of 60% in 74 tumors. Taylor [4] reported that cartilage is more frequent in tissues of the cervix than in those of the body of the uterus.

Regarding the origin of the hyaline cartilage, Perlstein [5] stated that it is from spindle cells. Hartfall [16] reported that it is from embryonic cells, and Kistler [17] suggested that it is formed by differentiation from pluricentric foci of cartilage tissues or embryonic connective tissues with the potentials of forming cartilage. The unique heterologous tissues are the striated muscle cell derivatives in mixed tumors. Lebowich and Ehrlich [18] found 12 corpus tumors with striated muscle cells. Taylor [4] reported that 13 of 40 tumors had striated muscle cells. Kulka and Douglas [19] illustrated the various stages from rhabdomyoblasts to well-developed striated muscle fibers. Since the striated muscle cells are derived from mesoderm, the presence of this element is a distinctive feature of the mixed mesodermal tumors of the female genital tract. Novak [20] considered their presence to be a diagnostic characteristic of these tumors. The other heterologous tissues are adipose and osteoid elements. These also are derived from mesodermal tissue, but the presence of these tissues has not been demonstrated regularly in a specific mixed tumor.

The tumor removed surgically from the patient of our report had the papillary cystadenocarcinoma and the sarcoma tissues mentioned. These histologic structures were more definite in the tissues obtained by necropsy. These latter tumor tissues had sarcoma cells, islets of atypical cartilage, atypical mesoblastic cells, and many immature striated muscle cells. The Fallopian tubes and the uterus had no tumor tissues.

Many hypotheses have attempted to explain the tissue origin of these mixed mesodermal tumors. Cohnheim's [21] hypothesis

that tumors develop from superfluous embryonic rests is not accepted by most pathologists. A modification of this theory was advanced by Wilms,[22] who postulated that the tumor arises from "rests" of the primitive mesodermal tissue of the lumbar region, displaced downward by the descent of the Wolffian duct, in which the tumor develops later. This concept seems to suffice to explain the development of the mixed mesodermal tumor in the female genital tract.

Before Wilms'[22] statement, Pfannenstiel[23] had proposed that the connective tissue of the uterus, by a process of metaplasia, could produce the various heterologous components found in the tumor. Nicholson[24] and Willis[34] elaborated this concept. Kehrer[8] proposed that displaced embryonic rudiments are associated with the Müllerian duct. Subsequently, Nicholson[24] suggested that the cells in the Müllerian duct might respond to some stimulus and produce the resultant tumor growth. On the other hand, Meikle postulated that the tumor arose in sites distant from the Gartner duct. To combine Kehrer, Nicholson, and Meikle's ideas, Meyer[25,26] stressed the nearness of the Müllerian and Wolffian ducts. Wilms'[22] theory was accepted by Mönckeberg,[30] Kehrer,[8] and Lahm.[27] Lebowich and Ehrlich[18] stated that the "cell rests" occur during the fusion of the Müllerian duct, the fusion line being near the ultimate anterior and posterior midsagittal planes of the uterus and vagina. Sternberg[3] believed that the neoplasm is derived from the Müllerian apparatus, and its basic tissue is the endometrial stroma, which is probably the least differentiated tissue of the Müllerian derivatives which persist in the adult. From it, the various carcinomatous and sarcomatous elements of the tumor evolve by a process of metaplasia conditioned by the embryologic potentialities of the Müllerian tissue. Taylor[4] suggested that the tumor originated from both epithelium and stroma of the endometrium, which are considered to be the primitive Müllerian mesoderm. The cancerous changes of the epithelium were believed to be secondary to the development of sarcoma. Nevertheless, the essential malignant tissues arise from stroma, and the heterologous tissues developed by a process of "aberrant differentiation."

The histogenesis of the mixed mesodermal tumors of the uterine appendages is considered to be the same as that of the uterus. Martin and Feroldi[29] reported a rhabdomyosarcoma of the ovary and suggested three possibilities of origin: by metaplasia, by dysembryoplasia, and by assumed multiple potentialities of differentiation of the ovarian mesenchyma. Sandison[12] concluded that these tumors of the ovary arise from embryonic tissues. MacFarlane and Pritchard[28] stated that the tumor originated from an endometrial cyst of the ovary derived from the Müllerian duct.

Mixed mesodermal tumors[31] and rhabdomyosarcomas[32,33] of the male urogenital tract may represent homologues of the mixed mesodermal tumors of the uterus and adnexa in the female. The mixed tumors of the male reported contain cartilage, muscle, and also epithelium; the rhabdomyomas, the atypical skeletal muscle cells.

The tumor of our report seems to have originated in the left ovary. The tumor removed surgically was pseudocystic, and had papillary adenocarcinoma tissues and masses of heterologous mesodermal atypical cartilage. The recurrent tumor tissues and the metastases had undifferentiated atypical cellular tissues, papillary or glandular carcinoma, plates of chondrosarcoma, and rhabdomyosarcoma, the cellular elements characteristic of a cancerous mixed mesodermal tumor.

Summary

A cancerous tumor of the left ovary of a Negro woman aged 58 had papillary and glandular carcinoma tissues, masses of atypical hyaline cartilage, and rhabdomyosarcoma cells, histologic structures that are characteristic constituents of cancerous mixed mesodermal tumors of the uterus and adnexa. The tumor was removed sur-

gically, but shortly thereafter symptoms of recurrent growth in the abdomen appeared. The necropsy demonstrated a large recurrence of the tumor in the pelvis, multiple metastases of the peritoneum, and a small glandular carcinoma metastasis of the left lung. This cancerous growth seems to be the first one reported as a mixed mesodermal tumor of the ovary.

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Hyperglycemia and Histochemical Changes Induced in Dogs by Clostridium Perfringens Toxin

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In a recent communication we reported [1,2] the development of hepatic glycogenolysis and an increase in serum inorganic phosphorus following the injection of Clostridium perfringens toxin in dogs. A more pronounced glycogen breakdown and elevation of blood glucose and inorganic phosphorus were noted in those animals subjected to a total pancreatectomy plus toxin. Dihydroergotamine injected prior to the toxin was accompanied by a delayed hyperglycemia and a delayed rise in inorganic phosphorus. Concomitantly, a reduction in the glycogen values for skeletal muscles was frequently observed, however, at the site of injection of the toxin a higher value was frequently noted.

In order to investigate the problems of glycogen transport and storage under these experimental conditions, we also performed a series of chemical, histochemical, and histologic examinations of the skeletal muscles, liver, kidney, and heart muscle. In this report we wish to present our findings and the possible biochemical mechanisms whereby the hyperglycemia is mediated.

Method of Study

All dogs were anesthetized with pentobarbital sodium (Nembutal). The C. perfringens toxin* was injected intramuscularly into the midportion of the hindleg of the animals (8 ml. per kilogram of body weight), and the leg was massaged in order to increase the rate of diffusion of the toxin. This was accompanied by the uniform development of local edema (at the site of injection) and shock, and as a rule the animals survived four to four and one-half hours. The striking disintegration of the renal mitochondria and reduction of the alkaline phosphatase previously noted were again observed in the kidneys of the animals receiving the toxin.

As previously reported, blood glucose [3] and inorganic phosphorus [4,5] determinations were performed prior to the injection of the toxin and at one-half hour, one hour, and hourly intervals subsequently. Glycogen determinations [6] were performed in duplicate on the liver and skeletal muscles prior to the injection of the toxin. At the termination of the experiment glycogen studies were performed on skeletal muscle obtained from the site of injection of the toxin, as well as on the skeletal muscle where no injection was given; sections of the liver and the muscle of the left ventricle of the heart were also studied for glycogen. In addition, the routine eosin-hematoxylin stain, Schiff's periodic-acid stain for glycogen, and stains for mitochondria and alkaline phosphatase were performed on these tissues and on sections of the kidney. Correlation of these data with the previously reported biochemical changes will be reviewed.

Four groups of dogs were studied. The control group consisted of four animals which received pentobarbital sodium alone and which were observed for the same period of time as the other groups. A second group consisted of eight animals which received the toxin alone. A third group, of four animals, received dihydroergotamine methanesulfonate (Dihydroergotamine; D. H. E. 45) subcutaneously (0.5 to 1.0 mg. per kilogram) one-half hour prior to the injection of the toxin. This dose of dihydroergotamine is 5 to 10 times the amount required to inhibit adrenal hyperglycemia for a period of six hours, but only one-fourth to one-half the amount required to inhibit epinephrine vasoconstriction. In the fourth group, of five animals, total pancreatectomy was performed prior to the injection of C. perfringens toxin.

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* The toxin was prepared and supplied by Eli Lilly & Company, Indianapolis.

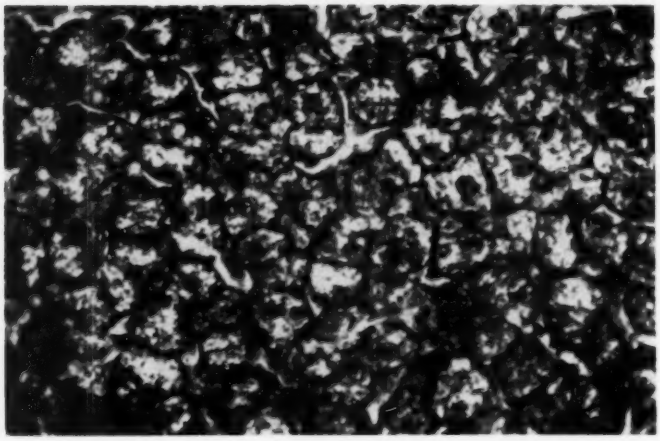

Fig. 1.—Liver from control animal (pentobarbital), showing normal-appearing mitochondria. Reduced to 62% of mag. × 800.

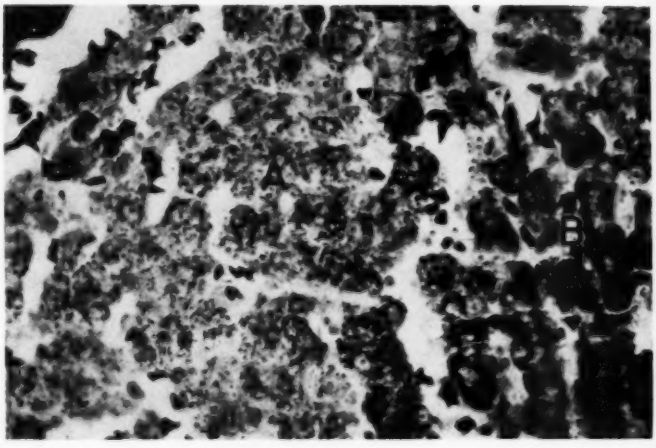

Fig. 2.—Liver removed four hours after administration of the toxin, showing the less severe alterations in the mitochondria (*B*) and the severer disintegration (*A*). Reduced to 62% of mag. × 800.

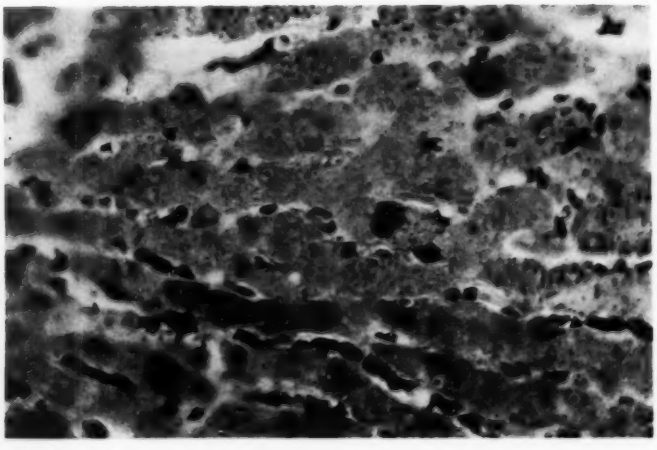

Fig. 3.—Same as Figure 2—liver removed four hours after the administration of the toxin, showing a more pronounced mitochondrial disintegration. Reduced to 62% of mag. × 800.

Distribution of Glycogen in Tissues in the Control and Toxin-Treated Dogs*

Tissue	Pentobarbital	C. Perfringens Toxin	Dihydroergotamine Plus C. Perfringens Toxin	Pancreatectomy Plus C. Perfringens Toxin
Liver (control)	--	3.3 ±3.0	1.78±0.8	1.69 ±1.4
Liver (end of experiment)	1.87±1.6	1.42±2.0	0.26±0.3	0.065±0.03
Muscle (leg) control	0.46±0.4	0.52±0.2	0.59±0.14	0.33 ±0.17
Muscle (leg) control (end of experiment)		0.46±0.4	0.27±0.26	0.26 ±0.17
Injected (leg) muscle		0.44±0.2	0.52±0.34	0.41 ±0.4
Heart muscles (left myocradium)	0.51±0.17	0.44±0.2	0.52±0.14	0.17

* Grams per 100 gm.

Experimental Observation

We have previously reported [1,2] that lethal doses of C. perfringens toxin injected intramuscularly into the hindlimb of dogs and the leg massaged produced hepatic glycogenolysis, with a rapid rise in blood glucose and without glycosuria. There was also a corresponding increase in serum inorganic phosphorus. In the animals that received pentobarbital sodium only, there was no appreciable effect on the serum glucose and inorganic phosphorus. In the animals that had a total pancreatectomy followed by injection of toxin there was a corresponding increase in the blood glucose. The distribution of glycogen in the tissues of these four groups of animals is summarized in the accompanying Table.

In the animals that received dihydroergotamine prior to the injection of the toxin there was no significant rise in the blood sugar and inorganic phosphorus for the first two hours; in the next hour there was a slight rise, followed by a more pronounced elevation of the blood glucose at the end of four hours.

The average of the control values for hepatic glycogen for all of the experimental groups was 2.28±2 gm. per 100 gm. When these values were compared with the control values for each individual experiment, a reduction in the glycogen content of the liver was observed in all of the animals receiving the toxin. The lowest values were observed in the pancreatectomy-plus-toxin group. From these chemical determinations it appears that the glycogen content of the uninjected skeletal muscles was lower at the end of the experiment than at the beginning, while the edematous muscle at the site of injection showed a slightly higher value. In the control group (pentobarbital alone)

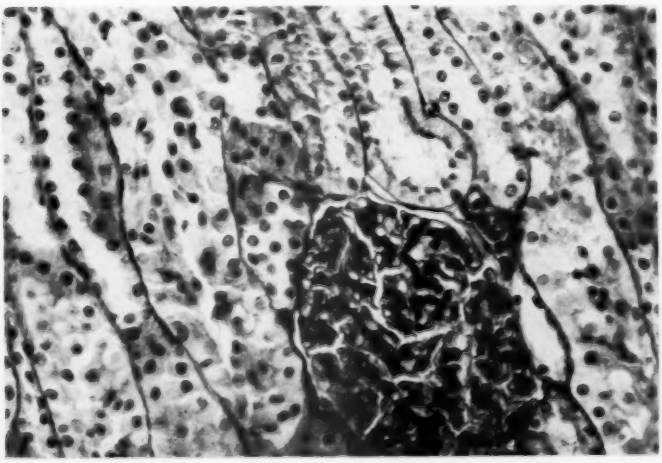

Fig. 4.—Kidney from control animal (pentobarbital), showing normal distribution of glycogen. PAS stain; reduced to 62% of mag. × 500.

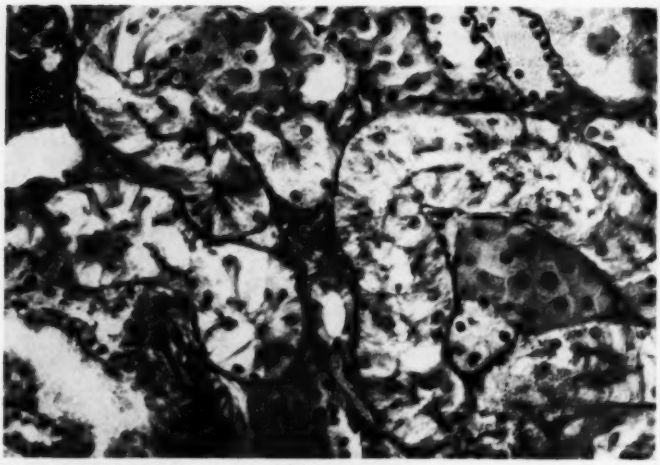

Fig. 5. — Kidney removed four hours after administration of toxin, showing a distinct diminution of glycogen. PAS stain; reduced to 62% of mag. × 500.

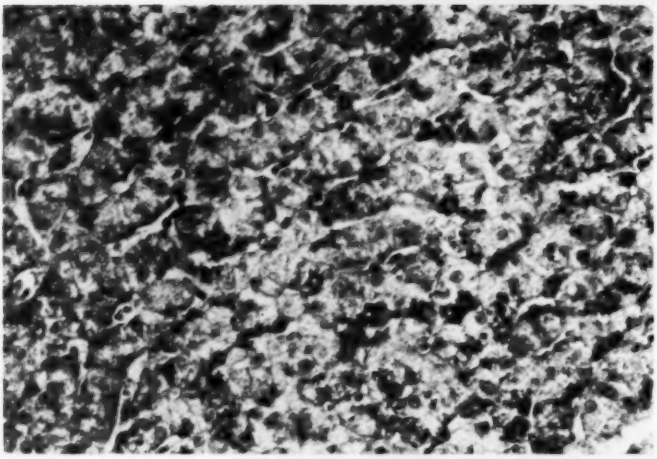

Fig. 6.—Glycogen in liver of control (pentobarbital) animal, showing a normal distribution of glycogen. Reduced to 62% of mag. × 300.

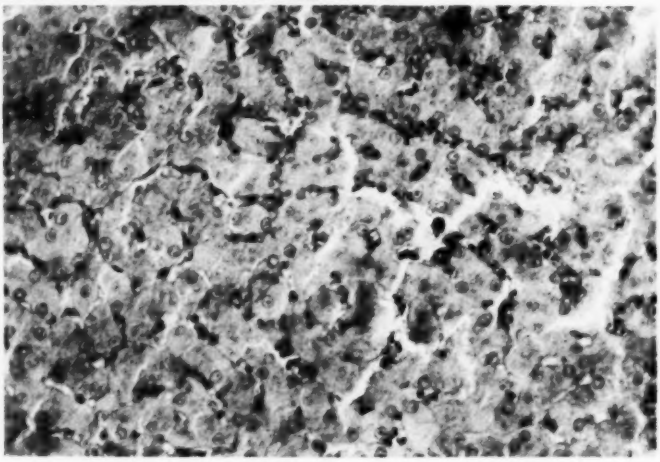

Fig. 7.—Liver removed four hours after administration of toxin, showing a diffuse reduction of glycogen. Reduced to 62% of mag. × 500.

there were no significant alterations in the glycogen values for liver and skeletal muscle at the end of four hours. The average glycogen contents of the cardiac muscle, in grams per 100 gm., were, respectively, 0.51 for the control group, 0.44 for the toxin group, 0.52 for the dihydroergotamine-plus-toxin group, and 0.17 for the pancreatectomy-plus-toxin experiment.

Histologic Observation

We were concerned with the alterations, if any, in the control and injected skeletal muscles, liver, kidney, and heart muscle, with reference particularly to the alkaline phosphatase, mitochondria, and glycogen changes.

Control Group.—In the control group the hematoxylin-eosin stains of the skeletal muscles, liver, kidney, and heart muscle did not reveal any remarkable changes except for minimal areas of congestion in the glomeruli, and this was an insignificant finding. The alkaline phosphatase of the tissues showed a normal distribution in the proximal convoluted tubules of the kidney and in the perilobular areas in the liver. The mitochondria in all of the organs were normal in appearance. The hepatic cells were filled with glycogen (each of the sections stained for glycogen also had a control study made with amylase to determine the effectiveness of the PAS stain). Glycogen was present in the kidney tubules, where it appeared somewhat more concentrated in the region of the brush border. The skeletal muscle and heart muscle also showed the presence of glycogen. Thus, the histologic appearance of these organs, the alkaline phosphatase activity, the distribution of the mitochondria and the glycogen-stained characteristics were within the normal range, while the blood glucose, the inorganic phosphorus, and the liver glycogen (chemical) showed little alteration during the four-hour period for this experiment.

Toxin Group.—C. Perfringens Toxin Only: Serial studies of hematoxylin-eosin-stained sections of the kidney demonstrated [7] a gradually increasing number of glomeruli showing vasoconstriction and swelling of the glomerular tuft, which was most pronounced between three and one-half and four hours after injection of the toxin. Between four and five hours after injection of the toxin there was wide dilatation of the glomerular capillaries, reduction in size of the tufts, and generalized vascular congestion, which was also observed in these experiments. Hemoglobin deposits were seen in several areas in Bowman's space, in some of the capillaries, and in the lumen of some of the tubules. Some of the tubules showed vacuolation, edema, and in some areas, absence of tubular membrane, a histologic observation which Oliver and his associates described [8] as tubulorhexis and which we have observed with severe toxic damage to the proximal convoluted tubules.

The hematoxylin-eosin control liver sections appeared normal; however, sections of the liver taken at the termination of the toxin experiment did show areas of vacuolation; some of the hepatic cells were swollen, and others appeared granular. The sinusoidal spaces, and in some areas in the peritubular zones the vessels, were dilated and filled with erythrocytes.

The hematoxylin-eosin studies of cardiac and skeletal muscles at the end of the experiment did not reveal any unusual changes. However, skeletal muscle taken from the site of injection of the toxin presented the histologic appearance of marked congestion, hemorrhage, and edema, as previously reported.

The distribution of the alkaline phosphatase in the kidney in these experiments was similar to what we have previously described,[9] namely, a marked destruction of the alkaline phosphatase in the region of the brush border of the convoluted tubules. There was a variable diminution of the alkaline phosphatase in the liver at the termination of the experiment, more pronounced in the peribiliary areas. The control muscle showed a minimal presence of this enzyme, and in the injected muscle

there was almost complete absence of alkaline phosphatase.

The mitochondria in the kidney, in general, showed severe and diffuse disintegration, particularly in the proximal convoluted tubules. Here, again, as we have indicated in our previous experiments,[7] the mitochondria in the proximal tubules in some cells were intact. Cells immediately adjacent showed marked disintegration of the mitochondria—in other words, an irregular and uneven appearance of the mitochondria from cell to cell in the renal tubules.

The distribution of mitochondria in the control sections of the liver was fairly uniform, many of the mitochondria having a regular rod-like or comma appearance, while others appeared as distinct granules filling the entire cytoplasmic area of the cell. At the termination of the experiment the following differences were noted: In many areas the mitochondria were smaller and altered in shape, presenting a granular appearance, with an occasional larger round granule. The mitochondria were reduced in number, and in many of the cytoplasmic areas no mitochondria were seen. In some experiments the alterations were less striking.

The mitochondria of the control skeletal muscle were evenly distributed throughout all of the cells. In the injected skeletal muscle there was an apparent patchy and uneven distribution of the mitochondria, many taking a paler stain, and in many cells they presented a granular appearance.

Sections of liver taken before the toxin was injected showed that glycogen could be demonstrated in fairly large quantities in all of the hepatic cells. At the termination of the toxin experiments there was noted a definite reduction in the glycogen content of the hepatic cells, which was diffuse throughout the entire liver sections. In some of the sections one could see small areas, particularly in the periportal zones, where there were increases in glycogen deposits. The uninjected skeletal muscle at the termination of the experiment demonstrated a reduction in glycogen. While many of the muscle bundles showed an intense glycogen stain, there were a greater number of poorly staining areas than in the biopsy control specimens of skeletal muscle taken at the beginning of the experiment. The injected leg muscle, which was edematous, hemorrhagic, and with many of the cells swollen, did show more glycogen distribution than in the opposite, uninjected leg muscle. There was no alteration in the glycogen distribution in the heart muscle. In the kidneys, reduction in the glycogen, particularly along the brush border of many of the cells of the proximal convoluted tubules, was noted; in others it was well preserved.

Dihydroergotamine plus Toxin: The hematoxylin-eosin, the glycogen, the alkaline phosphatase, and the mitochondrial sections were similar to those described in the toxin group. Similar findings were noted in the pancreatectomy-plus-toxin group.

Comment

The results of these experiments indicate three areas for analysis: (1) the role of hyperglycemia and elevation of inorganic phosphorus, in the wake of pentobarbital sodium, C. perfringens toxin injections, dihydroergotamine plus toxin, and pancreatectomy plus toxin; (2) glycogen determinations of control tissue specimens as compared with similar and other tissues at the termination of the afore-mentioned experiments; (3) histochemical studies of tissues for alkaline phosphatase, mitochondria, and glycogen, and the correlation of the chemical and histologic observations in these experiments.

There were no significant elevations in glucose and inorganic phosphorus in the pentobarbital, control dogs.

In the previous report we indicated that C. perfringens toxin alone resulted in a hyperglycemia of moderate degree within two to three hours without an accompanying glycosuria. When this was compared with the control for each of the experiments, an elevation of blood glucose almost twice the

control levels was found. We should like to comment at this point that the rise in blood glucose following toxin injection may be the result of C. perfringens toxin or its products on tissues or upon the enzyme systems of the latter. The liver and muscle, and at times the kidney, are predominantly affected.

Concerning C. perfringens toxin which was used in these experiments, the mechanism of the action of this toxin injected intramuscularly or intravenously into dogs appears to be a complex one, and probably represents a combination of factors.[10-14] There is a lecithinase component which acts on the phosphatides of cell surfaces, hemolyzing erythrocytes and affecting or destroying tissue cells, not only at the site of injection but at distant organs. This seems to be a most important factor, but there is also the combination with a hyaluronidase component (plus small amounts of unknown toxin) which enhances the formation of edema. There is also the possibility that some of the effects are not due to the direct action of the toxin alone but may be ascribed in part to the products of tissue breakdown.

In the experiments with dihydroergotamine and toxin the rise in blood glucose was slightly higher than in the pentobarbital experiment but less than with toxin alone and with pancreatectomy plus toxin. It is suggested that the dihydroergotamine injected prior to the toxin did have a delaying action on liver-glycogen breakdown in blocking the effect of epinephrine on the liver.

The rise in blood glucose in pancreatectomized dogs followed by injection of C. perfringens toxin was more pronounced than in any of the other experiments. The probable factor here was a more pronounced, shock-like effect and the absence of insulin in the accelerated glycogenolysis.

The inorganic phosphorus determinations paralleled the blood glucose curve. In the pentobarbital experiments there was no increase in the inorganic phosphorus. With the C. perfringens toxin alone, the dihydroergotamine plus toxin, and pancreatectomy plus toxin, the rise in inorganic phosphorus mirrored the pattern of the curves for blood sugar. In all of the toxin experiments there was a marked renal shutdown, with oliguria. In a study on the function of the kidney when tubular degeneration exists in lower-nephron nephrosis, Rice and Strickler[23] have shown that in many patients who died with tubular degeneration of the kidneys glucosuria was not present despite the high blood sugar level.

The toxins appear to have a potent in vivo effect upon mitochondria, producing alterations in size and shape, as well as extensive destruction, while affecting multiple organs. It has been shown by Witter and Cottone[15,16] that lysolecithin is a potent inducer of mitochondrial swelling in vitro and also that succinic oxidase, a mitochondrial enzyme, is highly sensitive to lecithinase.[17]

It may be of interest to report the observation of Porter[21] on the internal morphology of mitochondria. This author has shown that the variation among mitochondria of different cell types is greater than among cilia, but the general structural attributes are similar in all cases. Fisher and Gruhn[22] injected a potent anti-rat-kidney rabbit serum into rats and studied various enzyme and chemical alterations and compared these with untreated controls. There was a depletion of succinic dehydrogenase in the proximal and convoluted tubules as early as 18 hours following injection of nephrotoxic serum. There was a correlation of decrease of renal succinic dehydrogenase with alteration of mitochondria.

The evidence of enzymatic alterations from cell to cell seen in the hepatic cells and in the proximal convoluted tubules is striking. There may be complete destruction of the mitochondria in all of the cells lining the proximal tubules, or the mitochondria may be partially disintegrated in one cell and unaltered in the adjacent one. This applies to the glycogen and alkaline phosphatase. On the other hand, we have

previously shown[2] that the administration of dextrose prior to the administration of the toxin provided considerable protection for the alkaline phosphatase activity of the brush border of the cells of the proximal convoluted tubules, with no apparent protection against the destructive effect on the mitochondria of these cells. It was suggested[2] that the antagonism between dextrose and toxin with respect to the alkaline phosphatase activity is possibly competitive in nature at the site of combination with this cellular component of the resorptive mechanism. In a recent study on the transport of glucose by renal tubule cells employing C^{14}-labeled glucose, creatinine, and phlorizin,[18] it was suggested, on the basis of the reported findings, that glucose is transported without breakdown and without resynthesis of the six-carbon chain. The combination of glucose with the postulated intracellular carrier occurs at the cell barrier rather than within the cell and does not directly involve organized intracellular particles, such as mitochondria.

In correlating anatomic and functional changes, Oliver and MacDowell,[20] in their studies on renal lesions in epidemic hemorrhagic fever, have shown that patients recover from renal lesions in this clinical entity not by repair of their nephrons but because a great number of their nephrons have not been irreparably damaged. These authors are of the opinion that functional and organic alterations in renal activity are not similar. "They are in fact one and identical and no elaboration of technique can make them two." They further state: "If one can see a structural alteration, then the differentiation between the evanescent and the irreversible renal disturbance may be possible."

Thus, it can be postulated that there are organic changes in the enzymatic systems of the cells which are reflected in their functional activity. There is further evidence from the histologic studies of the organs, particularly liver and kidney, in which histochemical special stains for glycogen, mitochondria, and alkaline phosphatase were made, that there is not a complete destruction of these enzymes in all of the cells. Phenolsulfonphthalein (phenol red), which is secreted by the normal proximal convoluted tubules of the kidney[19] and observed in normal cells, was not found to be present in the cells at the end of the experiment, except for occasional scattered cells retaining apparently normal function.[7] This is true in animal studies and in man exposed to toxic agents and thus accounts for a bizarre alteration in various chemical and electrolyte changes, and may not necessarily imply damage to all the cells.

The hyperglycemia is apparently due to glycogenolysis of the liver, but there are also corresponding changes in other organs, particularly the kidney and skeletal muscles. This is reflected in the chemical and histochemical studies of these organs for glycogen. The control liver slices for glycogen were within the average normal range, and at the termination of the experiment there was a reduction in the chemical quantitative amount of glycogen. The control liver sections showed a normal distribution of glycogen with the PAS stain, and at the termination of the experiment there was a marked uniform reduction in the glycogen in the cells. There was not a total, or complete, absence of glycogen, as small deposits of glycogen were seen in many of the hepatic cells. This depletion of glycogen of the liver may be due to the effect on the enzyme system of the mitochondria or of the cytoplasm or a combination of the two. We did not have a control section of the kidney, but sections taken at the end of the experiment did show a diminution of glycogen.† The uninjected skeletal muscle at the end of the experiment appeared to have fewer deposits of glycogen as compared with the control; however, the injected skeletal muscle (toxin) was edematous, hemorrhagic, and swollen and contained a fair amount of glycogen, probably as a result

†We did have an opportunity to study kidneys from uninjected animals, and these were compared and were used as controls with the kidneys of noninjected toxin experiments.

of the edema in this muscle.‡ The heart muscle did not show any appreciable diminution of glycogen at the end of the experiment, and similar observations have been made by investigators in diabetes mellitus. Thus, it is suggested that hyperglycemia in the toxin-treated animals is derived essentially from the liver (glycogenolysis), with a relatively small amount from the kidney, and that there is a diminishing uptake of glycogen by the skeletal muscles; and this is in part reflected in the histochemical studies of these organs.

The histologic findings in the various organs in the dihydroergotamine and toxin experiments stained for glycogen by the PAS method were similar to the changes observed in the histologic studies in the experiments with toxin alone. The reduction of glycogen in the hepatic, kidney, and muscle cells was obvious, but it was difficult to differentiate these changes in the various experimental observations and under the conditions of the experimental studies.

Various factors may be considered to have a role in the mechanism of these changes. All of the animals received C. perfringens toxin (except the control, pentobarbital dogs). The severity and duration of the shock, tissue anoxia, and anaerobic breakdown of carbohydrates may be attributed in part to the direct effect of this toxic agent on tissue cells. This is accompanied by increased peripheral utilization of dextrose in the shocked animals, reduction in liver and muscle glycogen, a fall in body and limb temperature, and a depletion of high-energy phosphorus compounds, which are essential for the metabolism of carbohydrates. Packer and associates [24] have reported that changes induced by shock in various organs and tissues may be caused by anoxia and electrolyte imbalance, which is part of the shock syndrome. Swelling and shrinking of mitochondria cause changes in metabolic activity. In irreversible hemorrhagic shock Hift and Strawitz [25] are of the opinion that mitochondria accumulate abnormal amounts of phosphate, some of it in a form which is soluble in perchloric acid and reacts as inorganic phosphate under Fiske-Subbarow conditions.

C. perfringens toxin has a deleterious effect on the mitochondria of tissue cells, particularly of the renal tubules and hepatic cells. The alterations in mitochondrial morphology are reflected in the size and shape, as well as in the disintegration to destruction which affects the functional activity of the enzymatic system of the cells; but it is difficult to correlate the organic changes with functional activity. Since glycogen and alkaline phosphatase are an integral part with mitochondria of the enzyme structure of the cells, it is possible that a toxic agent which causes disintegration of one system (mitochondria) may exercise a corresponding change on other cytoplasmic enzymatic systems. The marked alteration of the mitochondria, glycogen, and alkaline phosphatase at the termination of the toxin experiment compared with the control, as demonstrated by histochemical studies, substantiates this observation.

Summary

Intramuscular injections of lethal doses of Clostridium perfringens toxin and massage, in order to increase the rate of diffusion, produced hyperglycemia, a concomitant increase in serum inorganic phosphorus, and oliguria to anuria without glycosuria.

The major factor in the resulting hyperglycemia was a hepatic glycogen breakdown. Chemical glycogen determinations of the liver revealed a marked reduction of glycogen. Histochemical examination of the liver showed a diffuse, as well as a marked, reduction of glycogen. A reduction in glycogen was also observed in skeletal muscle. A higher level of muscle glycogen was observed at the site of injection of the toxin. A slight reduction in glycogen of the kidney, more marked along the brush border, was

‡ Several factors may be considered for this observation, such as impaired circulation with vasoconstriction, hemoconcentration, anoxia, and the effect on the synthesis of lactic acid into glycogen.

noted. The heart-muscle glycogen was not appreciably altered.

The destructive effect of C. perfringens toxin upon the mitochondria of multiple organs was most striking in the kidney and liver.

The reduction in the alkaline phosphatase activity was more pronounced in the kidney than in the liver.

Pentobarbital sodium (Nembutal) alone did not have any appreciable effect on the blood biochemical studies and histologic alterations of the mitochondria or glycogen of the various organs.

Dihydroergotamine administered prior to C. perfringens toxin was accompanied by a delayed hyperglycemia and an apparent temporary delay in hepatic glycogen breakdown. Pancreatectomy plus injection of toxin resulted in a more pronounced elevation of blood glucose, and of inorganic phosphorus and in hepatic glycogen breakdown. The effect upon the mitochondria and alkaline phosphatase, as well as the glycogen of the liver and skeletal muscle, appeared to be similar to that observed for the toxin experiment alone.

Some of the possible mechanisms involved in the transport of glycogen in the production of the hyperglycemia are reviewed. The alterations in the cellular enzyme systems, as reflected in the striking morphologic alterations and disintegration of the mitochondria and the reduction in alkaline phosphatase and glycogen following the injection of C. perfringens toxin, are discussed.

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Tracheal and Bronchial Squamous Metaplasia in Influenza

Analysis of Fifty-Two Autopsy Cases

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The mention of squamous metaplasia in the mucosa of trachea and bronchi in influenza dates back to 1919, when Askanazy [1] described the autopsy findings of 90 patients dying of influenza. Thirty-eight of the latter displayed marked epidermidalization of bronchi.

During the same influenza epidemic of 1918-1919 similar changes in bronchi and trachea of patients dying of the disease were noticed by Glaus and Fritzsche [2] and Winternitz et al.[3] Recently, Werthemann [4] described epidermidalization of trachea and bronchi in influenza.

On the other hand, most of the other authors describing the pathological changes in influenza fail to mention squamous metaplasia of the respiratory epithelium (e. g., Wolbach and Frothingham,[5] Wegelin,[6] Miloslavich [7]).

The problem has been of interest to us because of the findings in experimental influenza in ferrets (Francis and Stuart Harris [8]) and mice (Straub [9] and Loosli [10]). Loosli [10] described marked destruction of bronchial epithelium in mice exposed to influenza virus A. The necrosis was evident 72 hours after infection, and first signs of regeneration by undifferentiated epithelium were seen seven days after infection. True squamous metaplasia was first observed 10 days after infection and reached its peak on the 14th day.

In human influenza the beginning of bronchial epithelial regeneration was seen by Hers [11] on the 3d day of illness, with a maximum activity on the 9th to the 15th day of the disease.

We have tried to analyze 52 postmortem cases that died of influenza in Basel during the last months of 1957 with regard to the incidence of squamous metaplasia of the respiratory epithelium.

Material

Histological sections from trachea, bronchi, and lungs from 52 influenza autopsies were studied. Of these, 37 were men and 15 were women. The tissue was fixed in 10% formalin, and the preparations stained with hematoxylin and eosin; in some instances special stains with the Van Gieson or Gram method were performed. On the average, two sections were taken from the trachea and three or four representative sections were taken from the lungs.

The diagnosis of influenza was arrived at on the basis of clinical history and findings (usually sudden onset of dyspnea and cyanosis), the fact that all these patients died during an influenza pandemic, and by virological examination in selected cases of lung tissue removed at autopsy, showing presence of the virus of influenza A (Asia). The diagnosis was also confirmed at autopsy by the finding of usually massive, occasionally focal pneumonia with a hemorrhagic and frequently necrotizing suppurative character. Very frequently necrosis and patchy desquamation of the tracheobronchial epithelium was seen.

In many cases there was secondary respiratory infection by hemolytic Staphylococcus pyogenes var. aureus or Diplococcus pneumoniae (pneumococcus).

The age and sex distribution of the patients are given in Chart 1.

Incidentally, in our material there is a preponderance of older people dying of influenza. This is in marked contrast with the findings during the influenza pandemic of 1918-1919, when most of the casualties were people in their 30's or 40's (Statistics of the Swiss Department of Health [12]).

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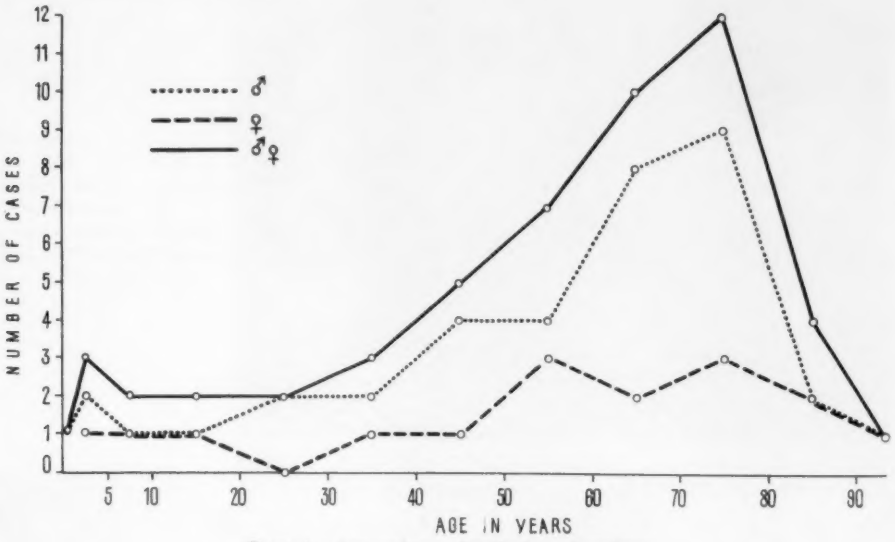

Chart 1.—Age and sex distribution of patients.

Microscopical Findings

The usual epithelium lining the respiratory tract in man is a ciliated pseudostratified columnar epithelium. As for the term metaplasia, we accept the definition given in Anderson's "Pathology" [13]: "Metaplasia is a form of abnormal regeneration in which is produced a type of cell different from the normally found in a given location." Squamous metaplasia or epidermidalization then implies replacement of the usual respiratory epithelium by squamous-cell epithelium.

Epidermidalization of the tracheobronchial epithelium was noticed in 27 out of 52 cases. It is possible that more cases of metaplasia would have been found had serial sections of trachea and bronchi been performed. In some cases the metaplastic changes were restricted to the trachea or the

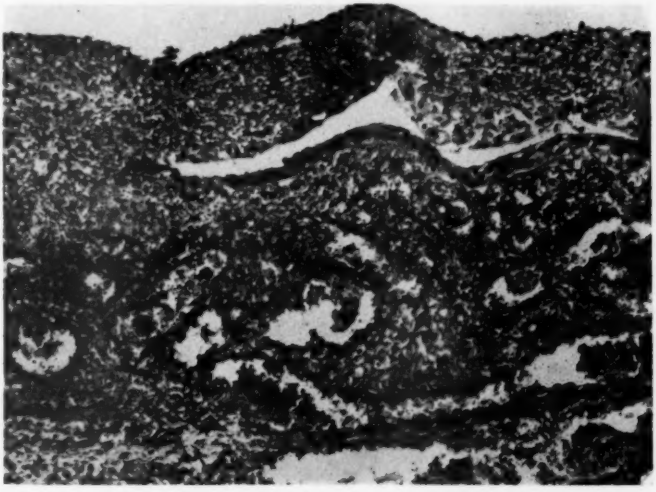

Fig. 1.—Trachea; 71-year-old man; sixth day of illness. Necrosis of epithelium, replacement by "diphtheritic" membrane, consisting of fibrin and trapped leukocytes. Under-membrane beginning epithelial regeneration by squamous epithelium. Hematoxylin and eosin; reduced about 10% from mag. × 270.

Fig. 2.—Trachea; 30-year-old man; 30th day of illness. Chronic tracheitis with squamous metaplasia. Van Gieson stain; reduced about 10% from mag. × 120.

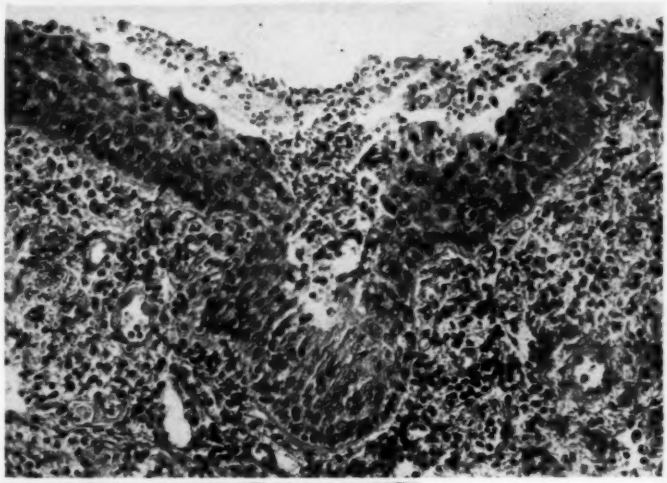

Fig. 3.—Trachea; 51-year-old man; second day of illness. Mucosa is replaced by squamous or undifferentiated epithelium. Chronic tracheitis. Exudate in lumen consists of erythrocytes, polymorphonuclear leukocytes, and fibrin. Hematoxylin and eosin; reduced about 10% from mag. × 270.

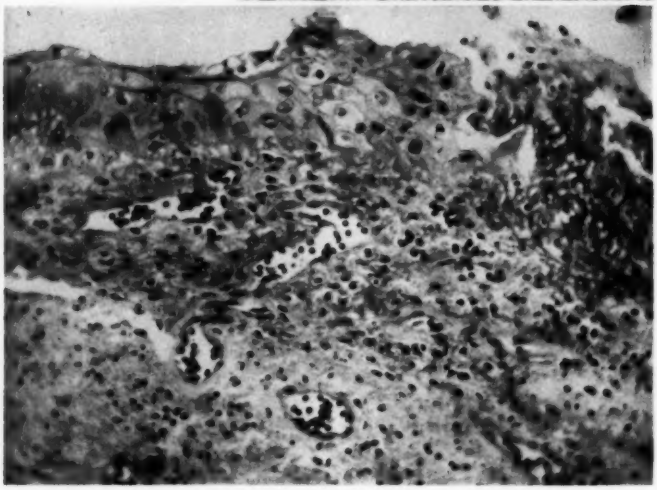

Fig. 4.—Trachea; 75-year-old man; second day of illness. Squamous metaplasia of epithelium. Diphtheritic necrosis of mucosa (in right upper corner of photograph). Hematoxylin and eosin; reduced about 10% from mag. × 270.

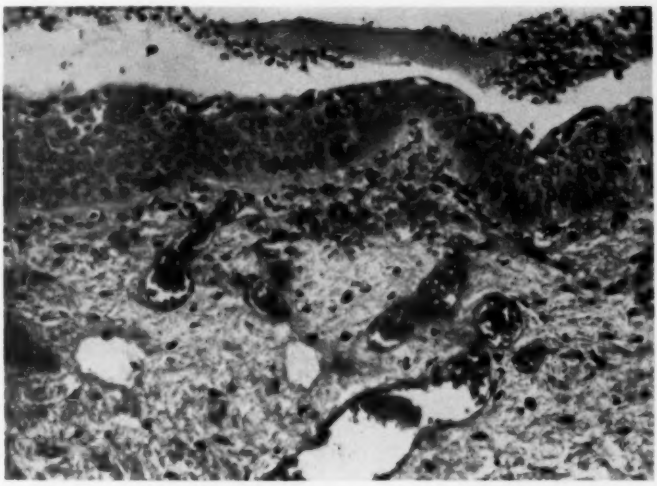

Fig. 5.—Trachea; 55-year-old man; seventh day of illness. Mucosa is replaced by squamous or undifferentiated epithelium. Hematoxylin and eosin; reduced about 10% from mag. × 270.

bronchi; in others metaplasia was found in both.

The earliest occurrence of squamous metaplasia was seen two days after onset of clinical signs of infection in three patients. In other areas of the respiratory tract or even in the same bronchi, concomitant epithelial necrosis and desquamation was taking place, and so the impression was obtained that necrosis and regeneration were sometimes occurring at the same time (Figs. 1 to 5).

Occasionally, the superficial layer of the ciliated epithelium desquamated *in toto* with preserved cilia. Some of these cells contained small cytoplasmic vacuoles (Fig. 6).

The remaining epithelium was reduced to one or two layers of cuboidal or flat cells or disappeared entirely. The regeneration was resumed from these islands of preserved basal epithelium.

Sometimes the areas of proliferation projected in a cushion-like fashion into the lumen of bronchi and in some cases they occluded the lumen entirely (Fig. 7). This obliterating bronchiolitis was not observed before the 16th day of illness in our series. Very frequently, this metaplastic hyper-

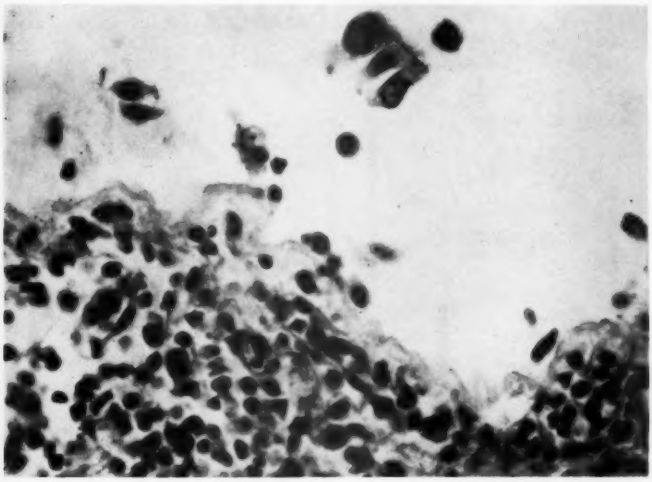

Fig. 6.—Lung, lumen of bronchus; 75-year-old man. Duration of disease unknown. Desquamated columnar respiratory epithelium with cilia in lumen of bronchus. Many vacuoles in cytoplasm of desquamated epithelium. Hematoxylin and eosin; reduced about 10% from mag. × 600.

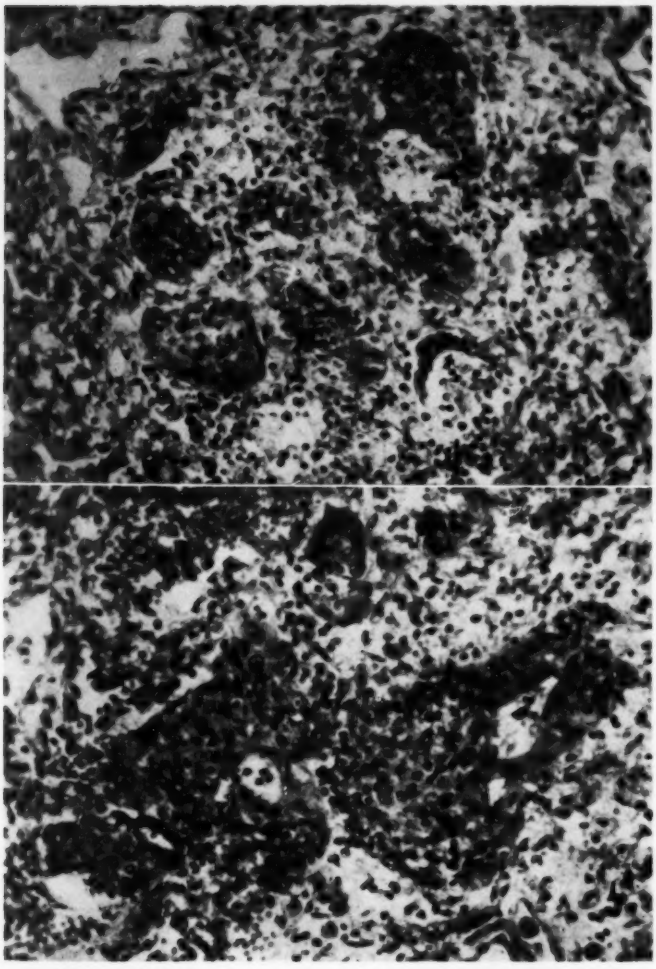

Fig. 7.—Lung; 17-year-old girl; 16th day of illness. Squamous metaplasia of bronchioli causing obliteration of lumen: obliterating bronchiolitis. Pictures are suggestive of spreading epidermoid carcinoma. Hematoxylin and eosin; reduced about 10% from mag. × 230.

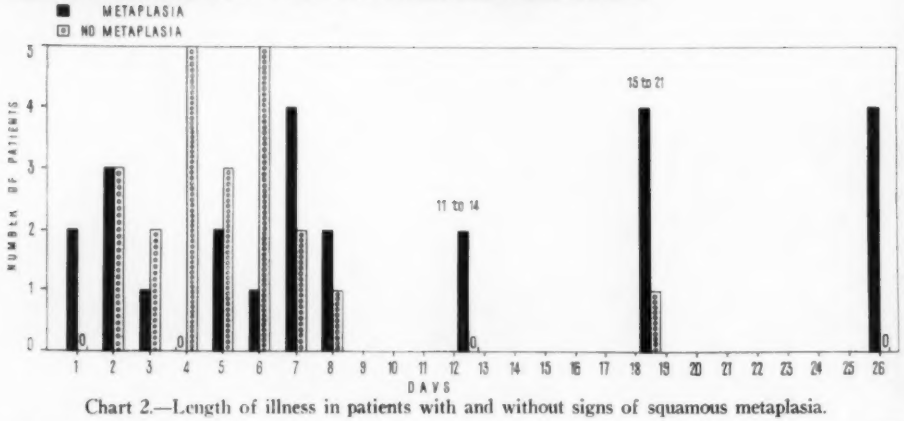

Chart 2.—Length of illness in patients with and without signs of squamous metaplasia.

plasia of the bronchiolar epithelium bore at first glance a striking resemblance to a spreading epidermoid carcinoma.

Epidermidalization was most commonly seen in patients dying after seven days of clinical illness or later: 16 of 23 cases of metaplasia, i. e., about 70%, died after seven days or later. On the other hand, 20 of 24 patients (80%) of those who died without evidence of metaplasia died within the first six days of illness. Chart 2 gives the relation of epidermidalization to the duration of the disease and also compares the length of illness in patients not displaying metaplasia of the respiratory epithelium.

We have asked ourselves whether advanced age and the possible concomitant lung pathology had anything to do with squamous metaplasia. However, we saw epidermidalization in a 2-month-old infant and in three other patients whose ages ranged between 11 and 30 years. It is true, though, that most cases were found in the older age group. This is probably colored by the fact that 65% (34 cases) of the patients dying of influenza belonged to the 50- to 90-year group.

Chart 3 gives the distribution of squamous metaplasia at various ages.

Out of the 52 cases studied, 37 were males and 15 were females, i. e., a ratio of approximately 2½:1. However, respiratory squamous metaplasia (that was seen in a total of 27 cases) appeared predominantly

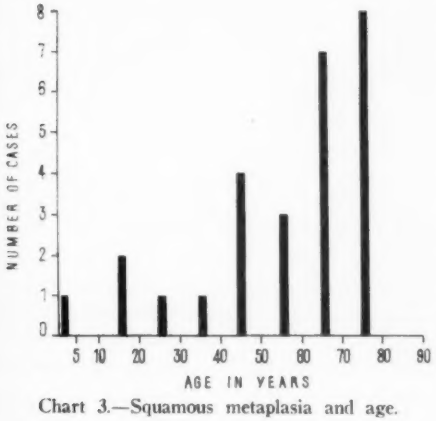

Chart 3.—Squamous metaplasia and age.

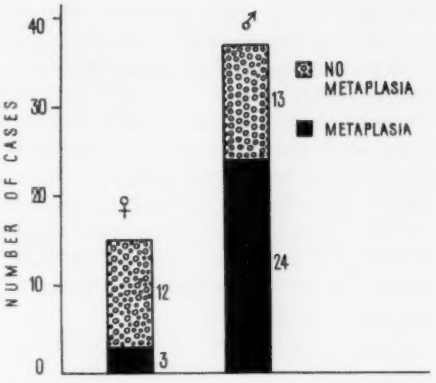

Chart 4.—Sex distribution of metaplasia in influenza.

in males, namely in 24 cases, and it was found in only 3 female patients. This results in a modified ratio of 8:1 (Chart 4). This increased occurrence of metaplasia in the male sex is of definite interest when reflecting on the well-known preponderance of the male in the incidence of bronchogenic carcinoma. Naturally, our series is too small to draw any valid conclusions.

Hyaline membranes in lung alveoli and alveolar ducts were frequently described in influenzal pneumonia. In our series they were seen but rarely and seemed to originate from the fibrin located in the alveolar lumen.

Also, a primary interstitial inflammation of the lung parenchyma, a pneumonitis, was not observed. The interstitial tissue was always invaded secondarily from the inflammatory focus originating in the bronchi or bronchioles.

Comment

Squamous metaplasia is probably preceded by an infection by the influenza virus of ciliated cells and other cells of the superficial layer of the mucosa, as demonstrated by the finding of virus particles or inclusions in these cells (Panthier et al.,[14] Harford and Hamlin,[15] Harford et al.[16]). It appears that the latter are desquamated intact into the lumen and serve as proliferating medium for the propagation of the virus.

The islands of basal epithelium that are spared form foci of regeneration.

Regeneration of the differentiated respiratory epithelium proceeds via undifferentiated transitional-cell type epithelium, latter squamous-cell epithelium that is eventually replaced by the usual pseudostratified columnar ciliated epithelium. In animal experiments complete regeneration by normal ciliated epithelium is seen three weeks (Francis and Stuart Harris [8]) to three months (Loosli [10]) after onset of illness.

It seems possible that in the animal remnants of metaplastic epithelium may persist after this time especially in the bronchioles (Straub [9]), but in man it is obviously difficult to ascertain whether such a late metaplasia is due to past influenzal or other infection or whether it has possibly been there even since birth.

Indeed, Weller,[17] in his analysis of 128 unselected patients ranging from a newborn to a patient of 86, noticed metaplasia even in healthy lungs; however, the incidence of bronchial epidermidalization was twice to three times as high in diseased lungs. Incidentally, metaplasia was almost twice as common in men as in women.

Bronchial squamous metaplasia is of course a well-known though not too common finding in chronic inflammation of the bronchi. Niskanen [18] mentions bronchiectasis, chronic fibrotic pneumonia, pulmonary tuberculosis, syphilis of the lung, chronic bronchitis, and bronchogenic carcinoma as conditions where squamous metaplasia of the respiratory epithelium may be found.

This opinion is reflected in textbooks of pathology. In Anderson's "Pathology" [13] the statement appears: "Metaplasia occurs under three conditions: chronic inflammation, vitamin A deficiency, or neoplasia." However, it is a fact that tracheobronchial squamous metaplasia is also found as a result of acute infections such as influenza.

Epidermidalization of the respiratory epithelium is also encountered in other acute infections caused either by a virus, e. g., in measles (McKenzie [19] and Brandt [20]), or by bacteria, e. g., in diphtheria (Goldzieher [21]).

Some workers have wondered about the role of secondary bacterial infection especially staphylococcal in the causation of epidermidalization. It is almost the rule that patients dying of influenza display a secondary bacterial infection, and our cases are no exception. Isolated cases of influenza without demonstrable concomitant infection have been described by Parker et al.[22] These authors do not mention squamous metaplasia of the respiratory epithelium, but their few cases died within the first three days of the disease, when epidermidalization is rarely seen.

The assumption that the primary affection of influenza resides in the respiratory epithelium is given some weight by the electronic microscopical examination of influenza-infected mice by Harford et al.[16] They describe influenza viruses in the cytoplasm of the ciliated and nonciliated cells situated in the uppermost layer of the respiratory epithelium. Inclusions reminiscent of those seen in other viral diseases were already observed in influenza in the tracheobronchial epithelium in mice by Panthier et al.[14] and Harford and Hamlin.[15]

It seems that animal and human influenza have much in common, and it is probable that epithelial changes in the respiratory tract in influenza are mainly caused by the viral infection. There is no doubt, however, that necrosis and destruction of the epithelium can be caused by secondary bacterial infection, especially staphylococcal; on the other hand, squamous metaplasia in staphylococcal pneumonias uncomplicated by influenza is very rare.

Another problem that has occupied many minds (Winternitz et al.,[3] Niskanen,[18] Wittekind and Strüder,[23] Weller,[17] Otto and Wagner,[24] Otto [25]) is the role played by squamous metaplasia of bronchi in the rising incidence of bronchogenic carcinoma. It was pointed out, too, that the pandemic of 1918-1919 was followed by an unusual rise of cases of bronchogenic carcinoma.

However, before incriminating influenzal changes in bronchi as a cause of bronchogenic carcinoma it would seem necessary to explain many contradictory facts:

The metaplasia in influenza represents a temporary replacement of the usual epithelium and is normally followed by a complete restitution, at least in experimental animals. Moreover, autopsies of people who in the past had suffered from influenza—a very large percentage of the population in the United States and Switzerland had had influenza in 1918-1919—fail to reveal bronchial metaplasia.

Conversely, histories of people suffering from bronchogenic carcinoma do not reveal, in many instances, influenzal infection in the past.

According to Wegelin,[26] the rise of lung carcinoma after 1920 is more due to an increase in undifferentiated carcinoma and to less extent to a rise of squamous-cell carcinomas; the latter would be expected if it had to follow influenzal epidermidalization of bronchi.

It is also hard to explain why lung carcinoma has increased following the influenza epidemic of 1918-1919 if there had been frequent epidemics since the 5th century; e. g., documented epidemics occurring in the 19th century were recorded in 1802, 1830, 1833, 1835, 1837, 1848, 1867, 1870, 1873, 1875, 1880-1881, 1886, and 1889-1890 (Widal[27]).

Lastly, no rise in lung carcinoma to parallel the increase in other countries has been observed up to 1950 in the little-smoking population of Iceland by Dungal,[28] in spite of a heavy influenzal morbidity in 1918-1919.

On the other hand, in our series tracheobronchial epidermidalization was eight times as common in males as in females. Could it be that the tendency of the bronchial epithelium to undergo proliferative changes as a response to injury is more inherent in the male than in the female sex?

Conclusions

Chronicity of infection is not the only prerequisite for epidermidalization of the respiratory epithelium. It seems that epithelial necrosis is also an important factor.

In our series squamous metaplasia of the respiratory epithelium appears more characteristic of influenzal infection in man than the presence of hyaline membranes and interstitial pneumonia.

Summary

Fifty-two postmortem cases of influenzal pneumonia are examined for the presence of squamous metaplasia of the respiratory epithelium. Epidermidalization was found in 27 cases, of which 24 were men. It was seen at all ages—ranging from 2 months to 79 years—but more so in the older age group. The relation of squamous metaplasia of influenza to bronchogenic carcinoma is discussed.

Professor Werthemann gave permission to study the above cases; Mr. Muspach drew the diagrams, and Mr. Sutter took the photomicrographs.

Manchester Memorial Hospital.

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Hepatic Fibrosis

Chemical and Histologic Studies During Subacute Ethionine Intoxication

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The processes, chemical and histologic, involved in the formation of fibers in the liver are still poorly understood. In recent years chemical and morphologic aspects of formation of connective tissue fibers have been extensively studied in several sites, such as induced granulomata,[1-4] skin wounds,[5,6] rabbit ear chamber,[7] and tissue cultures.[8,9] Fibrosis in parenchymal organs has received much less attention.[10] It appeared promising, therefore, to apply the principles involved in the studies of connective tissue to the liver in the state of active formation of new fibers. As a model of acute hepatic fibrosis, stages of the subacute ethionine intoxication in rats were selected.[11,12] Histologic alterations were compared with the content of amino acids characteristic of collagen and that of hexosamine as an index of mucopolysaccharide. In this study two fractions were obtained, one containing total collagen and the other soluble collagen, part of which apparently corresponds to procollagens.[13,14]

In the past, chemical determinations of collagen content in human and experimental (carbon tetrachloride and choline deficiency) cirrhosis were performed mainly by a gelatinization method.[15,16] Hydroxyproline as a measure of total collagen was used in studies of liver regeneration[17] and of ethionine-induced hepatic fibrosis and human cirrhosis.[18] In the latter study, a good correlation was noted between the hydroxyproline content and the amount of reticulum and collagen. Evidence was obtained that preformed hepatic reticulum contains a significant amount of hydroxyproline and that new formation of reticulum is associated with a further increase. The present studies are to expand these observations.

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Materials and Methods

Female Sprague-Dawley rats, 29 in number, with a body weight of about 200 gm. at the beginning of the experiment, were maintained ad libitum on a synthetic diet previously described[19] and subsequently modified.[12] The low-choline content as well as the particular vitamin content is essential for the development of a suitable lesion. The experimental group of 22 rats received this basal diet supplemented by DL-ethionine incorporated at a level of 5 gm. per kilogram of diet. Of the experimental animals, two were killed by cardiac exsanguination each week for nine weeks and four at the 12th week. Single control animals were killed at the 1st, 5th, 9th and 12th weeks; three, at the 7th week. The liver was removed immediately, one lobe fixed in neutral buffered 5% formalin and the remainder used for chemical study. Histologic studies were performed on sections stained with hematoxylin and eosin, Alcian blue,[20] chromatrope-2R aniline blue,[21] and Gomori silver impregnation, as well as periodic acid-Schiff (PAS) reaction after digestion with diastase (DPAS) and counterstaining with hematoxylin.[21] The polysaccharidal nature of the DPAS-positive material was confirmed by the disappearance of the color after acetylation and its return after deacetylation. Various structural features were graded arbitrarily (0-4+, in half units) by an observer who had no knowledge of the experimental history.

For chemical studies the tissue was quickly homogenized at 0 C in distilled water and purified according to Schneider.[22] The final residue was dried at 105 C overnight. This procedure removes

acid-soluble nitrogen-containing and carbohydrate materials, lipids, and phospholipids. The residue consists almost entirely of protein (its nitrogen content being about 15%), although glycogen and small amounts of other contaminants may be present. It will be subsequently referred to as fat-extracted dry liver (FEDL). All chemical results are expressed as content of this fraction. For further fractionation of this residue, a simplification of the procedure of Lowry et al.[29] was used. For extraction, 1 ml. of 0.1 N NaOH per 10 mg. was added to the FEDL and the mixture shaken overnight in the cold room at 2 C. After centrifugation, the supernatant was decanted and the residue shaken again with half the amount of 0.1 N NaOH for an additional four hours in the cold room. The suspension was again centrifuged and the combined supernatants filtered through Whatman No. 42 filter paper under suction and taken to a known volume with water. An aliquot of this solution was taken to dryness in vacuo. This fraction will be referred to as the soluble fraction.

The FEDL and the soluble fraction were hydrolyzed with 3 N HCl for eight hours in the autoclave at 18 lb. pressure. The solution was taken to dryness in vacuo over NaOH pellets, neutralized with NaOH, and quantitatively diluted to volume. Hydroxyproline was determined after H_2O_2 oxidation and conjugation with *p*-dimethylaminobenzaldehyde (according to Neuman and Logan,[34] as modified by Martin and Axelrod[35]), proline after reaction with ninhydrin in strongly acidic solution (following Troll and Lindsley[36]), and glycine by ninhydrin oxidation to formaldehyde, the latter being reacted with chromatropic acid after distillation (according to Alexander et al.[37] as modified by Christensen et al.[38]) Hexosamine was determined by reaction with indole before and after deamination, following deacetylation and hydrolytic cleavage of the polysaccharides by heating for three hours with 2 N HCl at 100 C (following Dische and Borenfreund,[39] as described by Ashwell[40]). The protein content of the soluble fraction was determined by ultraviolet spectrophotometry (according to Warburg and Christian[41] as described by Layne[42]), which method depends primarily on the absorption of tryptophan and tyrosine at 280 mμ.

Results

Histologic Observation.—The basic lesion seen after eight weeks of administration of an 0.5% ethionine-containing diet consisted of a diffuse degeneration of the hepatic cells with variable clumping and vacuolation of the cytoplasm. The liver-cell plates were disarranged without abolition of the lobular architecture. In the cytoplasm, DPAS-positive granules accumulated. Single liver cells were necrotic; others showed conspicuous regeneration. These hepatocellular lesions were associated with a conspicuous accumulation of cells between the liver-cell plates, which frequently seemed to compress the sinusoids. These interstitial cells consisted of organized and disorganized-appearing ductular cells intermixed with lymphocytes, histiocytes, and segmented leukocytes. The Kupffer cells were moderately increased in number as well as enlarged, their cytoplasm containing DPAS-positive granules of varying size. Some of these were very deeply colored and represented lipofuscin, as verified by their brown fluorescence. The border between liver cells and the capillaries, only faintly visible in control animals, exhibited a strong DPAS reaction, also positive in Alcian blue stain. The portal tracts were enlarged, and contained many ductules surrounded by inflammatory cells and lipofuscin-carrying macrophages. In silver impregnations, the reticulum fibers were conspicuously increased. This was only to a small part caused by collapse following necrosis of single liver cells. More frequently the increase of reticulum fibers was the result of duplication of preformed layers around sinusoids or of basement membranes around the newly formed interstitial ductules. In chromatrope-2R aniline blue stains, only an insignificant increase of thick blue membrane was observed, which enforced the sinusoidal walls or radiated from the portal tract. The described changes developed at various speeds. The DPAS-positive material on the sinusoidal border of the liver cells, in Kupffer cells, as well as in the cytoplasm of the liver cells, was already as marked after the third week as in the eighth week. In contrast, the amount of reticulum fibers was at this time not increased; however, the arrangement was somewhat altered because of irregular direction of the liver-cell plates. From the seventh week on, however, a marked increase of the reticulum fibers was apparent (Figs. 1-3).

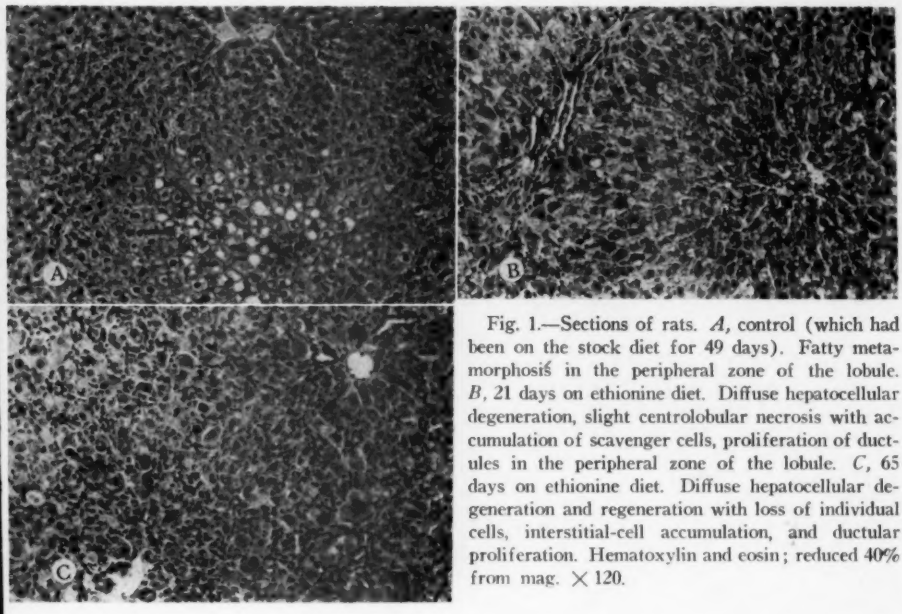

Fig. 1.—Sections of rats. *A*, control (which had been on the stock diet for 49 days). Fatty metamorphosis in the peripheral zone of the lobule. *B*, 21 days on ethionine diet. Diffuse hepatocellular degeneration, slight centrolobular necrosis with accumulation of scavenger cells, proliferation of ductules in the peripheral zone of the lobule. *C*, 65 days on ethionine diet. Diffuse hepatocellular degeneration and regeneration with loss of individual cells, interstitial-cell accumulation, and ductular proliferation. Hematoxylin and eosin; reduced 40% from mag. × 120.

After 12 weeks the regenerative changes had proceeded to the formation of small nodules composed of large liver cells without ductular cells and little reticulum stroma in the vicinity. In the surrounding parenchyma, the previously described changes were aggravated. The control animals showed, except for mild fatty metamorphosis, no significant abnormalities.

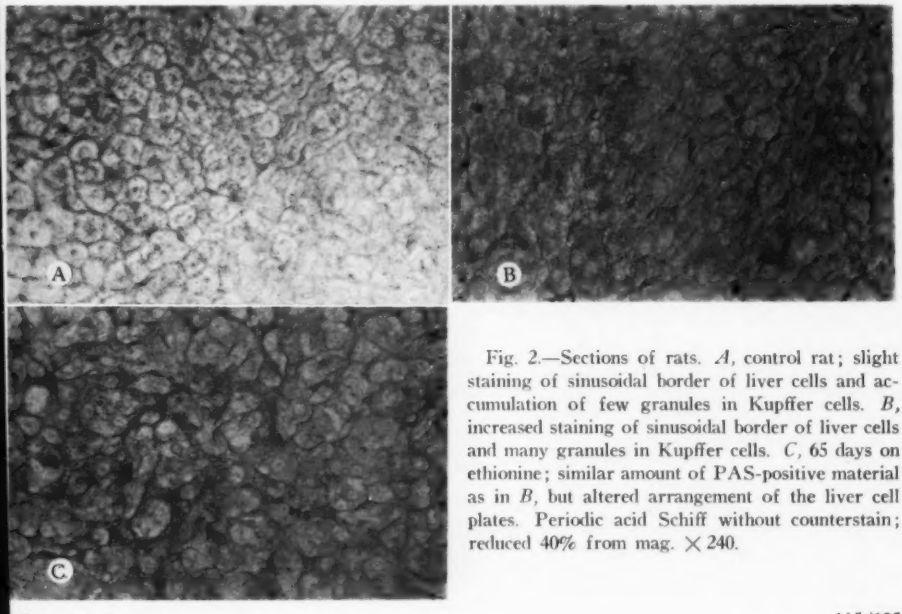

Fig. 2.—Sections of rats. *A*, control rat; slight staining of sinusoidal border of liver cells and accumulation of few granules in Kupffer cells. *B*, increased staining of sinusoidal border of liver cells and many granules in Kupffer cells. *C*, 65 days on ethionine; similar amount of PAS-positive material as in *B*, but altered arrangement of the liver cell plates. Periodic acid Schiff without counterstain; reduced 40% from mag. × 240.

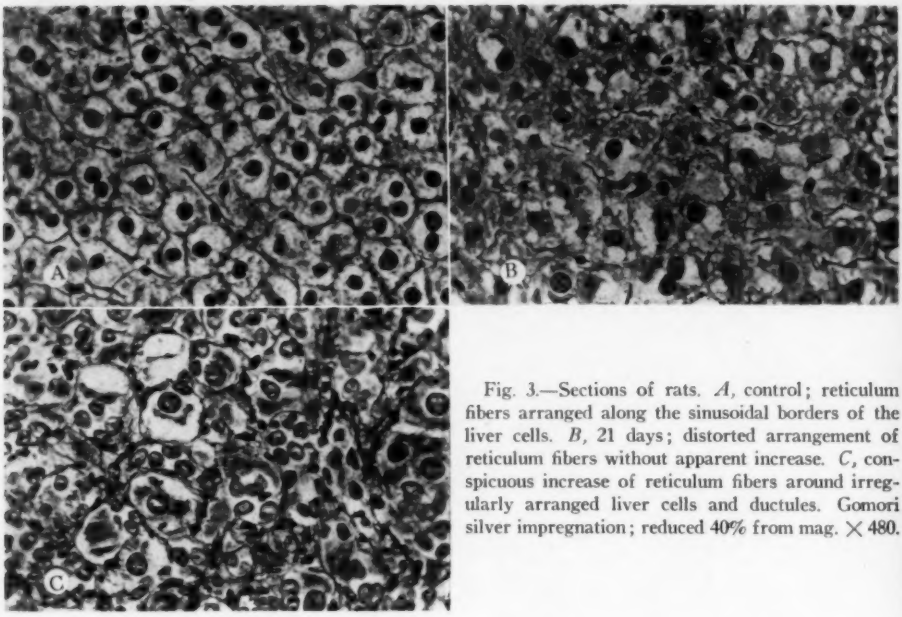

Fig. 3.—Sections of rats. *A*, control; reticulum fibers arranged along the sinusoidal borders of the liver cells. *B*, 21 days; distorted arrangement of reticulum fibers without apparent increase. *C*, conspicuous increase of reticulum fibers around irregularly arranged liver cells and ductules. Gomori silver impregnation; reduced 40% from mag. × 480.

The degree or amount of the following criteria was estimated during the course of the ethionine intoxication: (*a*) liver-cell damage, (*b*) ductular-cell proliferation, (*c*) reticulum by argyrophilia, (*d*) collagenous fibers and membranes by chromatrope-2R aniline blue staining, (*e*) polysaccharide material in the Kupffer cells by DPAS staining, (*f*) total acid mucopolysaccharides by Alcian blue staining, and (*g*) polysaccharide material on the liver-cell sinusoidal border by DPAS staining.

Chemical Results.—During the course of the experiment, the total protein-bound hydroxyproline in livers of the control rats showed fairly constant values around 0.8 mM. per 100 gm., which corresponds to 0.10 gm. per 100 gm. of FEDL (Table 1).

TABLE 1.—*Hydroxyproline, Proline, and Glycine Content of Dry, Purified, Defatted Liver and Percentage of Total Amount of These Components in Fraction Soluble in 0.1 N NaOH* *

		Hydroxyproline		Proline		Glycine	
Wk. on Diet	Rats, No.	Total, mM/100 Gm. FEDL	Soluble, % of Total	Total, mM/100 Gm. FEDL	Soluble, % of Total	Total, mM/100 Gm. FEDL	Soluble, % of Total
Controls	7	0.79±0.13 †	19.4±1.9	39.4±1.1	20.6±1.8	59.2±3.2	17.7±1.4
1	2	1.15±0.09	19.8±1.9	41.7±0.5	18.6±0.6	60.3±3.0	18.3±1.0
2	2	0.88±0.07	19.8±0.4	39.6±0.5	19.6±1.3	58.2±8.0	24.3±3.1
3	2	0.93 ‡	21.0	38.0	16.5	67.4	18.5
4	2	1.10±0.06	21.0±1.8	39.2±1.1	26.4±0.6	66.1±0.5	18.1±1.3
5	2	1.05±0.03	22.3±0.2	39.1±2.3	31.8±0.8	57.8±6.1	21.5±0.3
6	2	1.15±0.02	21.0±1.9	41.4±0.3	20.0±0.4	54.5±0.6	18.6±1.7
7	2	2.09±0.02	31.4±1.7	36.2±1.0	26.4±0.6	62.6±1.3	24.7±0.3
8	2	2.62±0.40	42.6±1.6	39.6±1.3	23.9±0.0	75.0±3.9	22.9±1.9
9	2	2.59±0.36	42.7±1.7	39.4±2.0	21.3±3.1	61.4±1.4	29.8±0.9
12	4	2.32±0.12	38.9±2.1	40.0±0.2	19.3±1.3	65.7±1.8	18.8±2.1

* FEDL indicates dry, purified, defatted liver.

† Means ± S. D., which for 2 samples is equivalent to Means ± Av. D.

‡ Only 1 animal analyzed for these components; see, however, "Protein," Table 2.

Of this, approximately 20% was in the soluble fraction. In the experimental animals, a small and irregular rise of the total hydroxyproline occurred at the onset of the experiment, lasting through the sixth week without significant variation in the distribution between the soluble and insoluble fractions. From the seventh week on, much higher values of the total hydroxyproline content were noted, and in the seventh and eighth weeks, values of more than three times the normal were recorded. Simultaneously, the percentage of the hydroxyproline in the soluble fraction rose to more than 40%, twice that of the original. This implies that the hepatic content of soluble hydroxyproline rose approximately 7.3 times, whereas that of the insoluble hydroxyproline rose 2.3 times.

The proline content of the FEDL was approximately 40 mM. per 100 gm., which corresponds to 4.5 gm. per 100 gm. This value did not undergo any significant changes during the entire course of the experiment. The percentage in the soluble fraction, however, rose significantly in the 4th and 5th weeks to about 1.5 times that of the control, and gradually returned to the control value in the 9th and 12th weeks.

The total glycine concentration of the FEDL was approximately 60 mM. per 100 gm., corresponding to a value of 4.5 gm. per 100 gm. It was somewhat elevated in the third and fourth weeks, but a really marked peak developed in the eighth week.

TABLE 2.—*Hexosamine Content of Dry, Purified, Defatted Liver and Percentage of Total Amount of Hexosamine and Protein in Fraction Soluble in 0.1 N NaOH* *

Wk. on Diet	Rats, No.	Hexosamine: Total mM/100 Gm. FEDL	Hexosamine: Soluble, % of Total	Protein, Soluble, % of Total
Controls	7	1.65±0.28 †	7.9±2.8	26.2±6.9
1	2	1.51±0.20	7.3±2.0	24.0±0.4
2	2	1.81±0.03	11.1±0.4	27.9±0.4
3	2	1.89 ‡	13.8	27.9±2.9
4	2	1.40±0.03	18.4±2.0	26.8±2.5
5	2	1.82±0.12	12.3±1.1	25.2±2.2
6	2	2.43±0.15	8.8±0.7	27.9±0.1
7	2	2.58±0.00	15.2±1.8	34.1±3.1
8	2	2.50±0.06	16.7±1.2	47.5±4.0
9	2	2.67±0.21	8.6±1.7	39.2±0.2
12	4	2.44±0.11	11.0±1.3	42.2±6.4

* FEDL indicates dry, purified, defatted liver.
† See †, Table 1.
‡ See ‡, Table 1.

At this time the percentage in the soluble fraction was not significantly changed.

The total hexosamine concentration of the FEDL was 1.65 mM. per 100 gm., corresponding to approximately 0.3 gm. per 100 gm. (Table 2). Of this, only about 8% was in the soluble fraction. The total hexosamine increased from the sixth week on. From the seventh week on, the levels were approximately 60% higher than the average control value, while the percentage in the soluble fraction was not altered.

Initially, about one-fourth of the total protein was alkali-soluble. A distinct increase to one-third occurred in the seventh week, to reach almost one-half of the total protein in the eighth week.

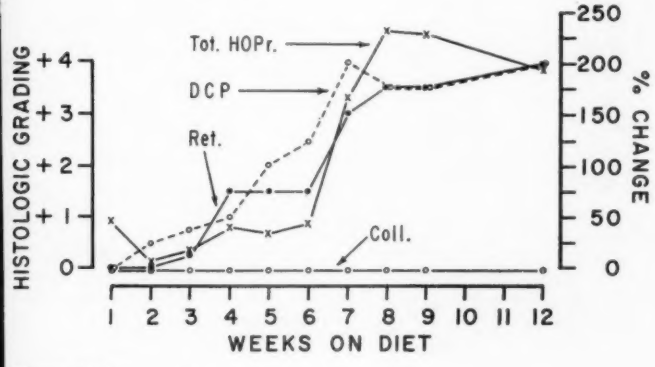

Fig. 4.—Alterations in reticulum (*Ret.*) and collagen (*Coll.*) content and in ductular-cell proliferation (*DCP*), graded histologically, and percentage change from controls of the total hydroxyproline (*Tot. HOPr.*), determined chemically, in livers of rats fed a diet containing 0.5% DL-ethionine.

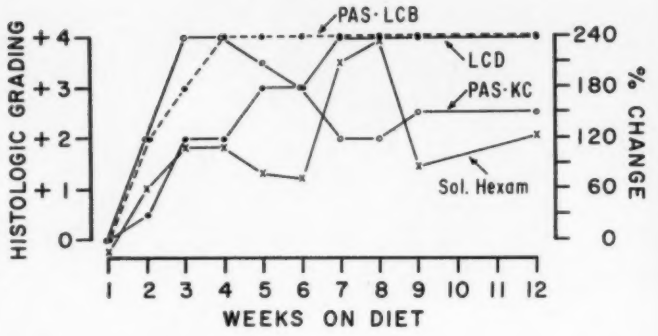

Fig. 5. — Changes in liver-cell damage (*LCD*), and in the PAS staining (after diastase) on the liver-cell/sinusoidal border (*PAS-LCB*) and in the Kupffer cells (*PAS-KC*), graded histologically, and percentage change from controls of the hexosamine soluble in 0.1 N NaOH (*Sol. Hexam*), determined chemically, in livers of rats fed a diet containing 0.5% DL-ethionine.

Correlation Between Histologic and Chemical Findings.—The conspicuous increase in reticulum and in the ductular-cell proliferation paralleled the rise of the total hydroxyproline (Fig. 4). No significant collagen increase, however, was recorded.

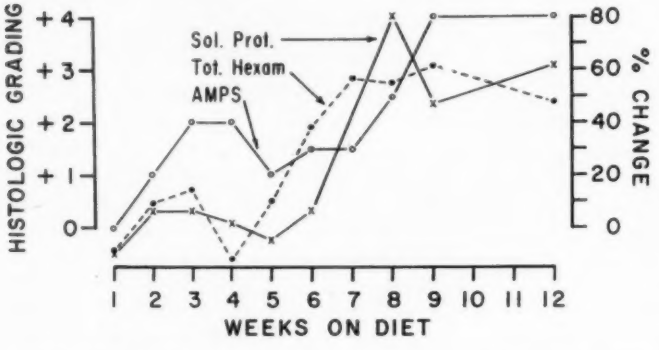

Fig. 6.—Alterations in acid mucopolysaccharide (*AMPS*) content, graded histologically, and percentage change from controls of chemically determined total hexosamine (*Tot. Hexam*) and 0.1 N NaOH-soluble protein (*Sol. Prot.*) of livers of rats fed a diet containing 0.5% DL-ethionine.

The PAS-positive material on the liver-cell border and in the Kupffer cells rose rapidly within the first three weeks (Fig. 5). At this time also, the soluble hexosamine started to rise and the liver-cell damage developed; however, these latter two kept increasing up to the seventh week. The acid mucopolysaccharide rose more slowly, with concomitant increase in the total hexosamine (Fig. 6). The liver-cell damage is also reflected, to a moderate degree, in the increase of soluble protein.

If the chemical figures were compared with each other by use of the absolute concentration of the various amino acids in the FEDL and soluble fraction (Fig. 7), the first rise occurred in the soluble hexosamine fraction in the third week and sub-

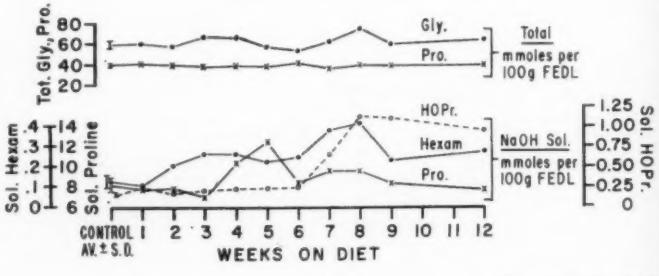

Fig. 7. — Variations in the content of total glycine (*Tot. Gly.*) and proline (*Tot. Pro.*), and of the 0.1 N NaOH-soluble hydroxyproline (*Sol. HOPr.*), hexosamine (*Sol. Hexam*) and proline (*Sol. Proline*) during ethionine intoxication of rats, expressed as millimoles per 100 gm. of fat-extracted dry liver (*FEDL*).

sequently in the total hexosamine. This is followed in the fourth to fifth weeks by a rise of proline in the soluble fraction, while the total proline did not undergo any significant changes. The total and soluble hydroxyproline rose almost simultaneously (seventh week), that in the soluble fraction being twice as marked as that in the total. Content of glycine rose in both fractions slightly later than that of hydroxyproline.

Comment

Any interpretation of the data presented has to be cautioned by the fact that the fractions contained an admixture of parenchymal proteins and therefore a lower concentration of scleroproteins (collagen and reticulum) than similar fractions obtained from connective tissue. Nevertheless, the surprisingly good correlation with histologic observation renders some credence to the interpretations.

In confirmation of previous studies,[18] a conspicuous increase of reticulum fibers is found associated with a parallel increase in hydroxyproline content. Since the histologically demonstrable collagenous fibers and membranes hardly multiply, it is safe to assume that the hepatic reticulum contains considerable amounts of hydroxyproline, again in confirmation of the study mentioned. This increase in hydroxyproline is proportionately greater in the alkali-soluble than in the insoluble fraction. In the former it rises to more than seven times its original value while in the latter it is elevated less than two and one-half times.

The studies of Orekhovitch[13] have suggested the existence of a procollagen, characterized by its solubility in citrate buffer. Harkness et al.[14] have supported this concept, using extraction by alkaline phosphate buffer. The NaOH-soluble fraction employed in the present work would include the "procollagen" fraction of the above investigators and in addition allow for measurement of soluble scleroprotein. The increase observed in the scleroprotein content of the soluble fraction during ethionine intoxication may reflect an increase in the procollagen components of the liver. Recently it has been suggested that the difference between procollagen and collagen is age rather than chemical constitution.[33] In this light, the conspicuous increase in both fractions, though two and one-half times as marked in the procollagen-containing fraction, would indicate a rapid aging of collagen during formation. Collapse and approximation of preexisting collagen, though seen histologically, would not be sufficient to account for this increase.

The sharp increase of hydroxyproline, particularly that in the soluble fraction, and of the reticulum fibers, which occurs in the seventh week of this study, invites consideration of its relation to the other biochemical and histologic features. Histologically, the ductular-cell increase exhibits the closest correlation, suggesting an actual relationship between fiber formation and proliferation of ductular cells. Previously, the opinion has been expressed[34] that the ductules stimulate fiber formation in the form of basement membranes, for which the ductule acts as a mold. Liver-cell damage distinctly precedes these changes. It is associated with an increase of DPAS-positive material on the sinusoidal border of the liver cells as well as in the Kupffer cells. Histochemical reactions, such as the sensitivity to diastase and acetylation, suggest that this material is a neutral mucopolysaccharide. The relation of this increase in neutral mucopolysaccharide around liver cells and in Kupffer cells to the liver-cell damage with which it coincides in various human and experimental liver injuries is the subject of histochemical analysis presented elsewhere.[34] Whatever the exact nature of the compounds in question is, polysaccharide appears around the liver cells and in the Kupffer cells before an increase of reticulum occurs. Hexosamine increase precedes hydroxyproline rise. Fiber formation elsewhere in the body takes place after a polysaccharidal matrix has been laid down, as demonstrated by histochemical and chemical techniques.[35,36] A similar process may take place during fiber formation in the

liver. It is not certain whether the polysaccharidal material is formed or phagocytosed by the Kupffer cells, though the latter seems to be the case.[34]

The interpretation of the variation of the proline and glycine concentrations in relation to the hydroxyproline increase and scleroprotein formation is more tenuous because of the large amount of cellular protein in both fractions. Collagen contains 14% proline and 28% glycine, while hepatic tissue has only 4.5% of each. Nevertheless, assumption of a relation of the changes of the amino acid concentration of the fractions to fiber formation deserves confirmation on fractions relatively much richer in collagen. While the total proline content does not vary, the soluble fraction exhibits a significant transient rise in the fourth and fifth weeks. One may speculate that proline of the liver cells moves from the insoluble to the soluble fraction and, it must be noted, at a time when no increase in soluble protein occurs. The relation between the curves of proline and hydroxyproline in the soluble fraction suggests a precursor relationship, in keeping with the studies of Stetten and Schoenheimer[37] and Stetten,[38] who found that proline formation precedes and is directly related to hydroxyproline formation, and with those of other investigators,[39,40] who suggested an intrachain oxidation of proline to hydroxyproline.

These features suggest that after formation of the carbohydrate matrix proline appears in the soluble fraction as a source for the formation of hydroxyproline and hence of scleroprotein. The third amino acid found in high amount in scleroprotein, glycine, shows indeed a different behavior in that it rises in both soluble and insoluble fractions. This suggests a participation of extrahepatic glycine, presumably coming from the blood, in fiber formation. The fact that the soluble protein rises at the time of fiber formation is remarkable and may be a reflection of tissue breakdown. Again, use of better-purified collagen fractions should clarify the relation between the rise in soluble protein and the simultaneous rise of soluble protein-bound hydroxyproline.

Summary and Conclusions

To study fiber formation in the liver, hydroxyproline, proline, glycine and hexosamine were chemically determined in fractions of hepatic tissue during the course of the subacute ethionine intoxication of the rat and correlated with histologic features. The increase of reticulum was associated with an increase of ductules and of hydroxyproline as chemical evidence of scleroprotein formation. A considerably increased amount of hydroxyproline was found in an alkali-soluble fraction which would include the procollagen-containing fraction described in the studies on connective tissue-rich materials (e. g., skin). The increase of histologically demonstrable acid and neutral mucopolysaccharides, as well as of chemically determined hexosamine, preceded the increase in hydroxyproline and reticulum. This suggests that these polysaccharides serve as matrices for fibers, in similarity to other tissues, the more so since they appear in the area where fibers form. While the total proline content did not rise, a transient rise did occur in the proline fraction that is alkali-soluble. Both total and soluble glycine rose subsequently. Pending further confirmation on better-purified fractions, it is suggested that liver-cell injury is associated with the appearance of polysaccharides which act as a matrix for procollagen and collagen. It is further suggested that there is a transfer of proline, which serves as precursor for hydroxyproline, from the liver cells to the procollagen, while glycine is contributed by the bloodstream.

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News and Comment

ANNOUNCEMENTS

Fellowships in Forensic and Anatomic Pathology.—The City of Philadelphia, Medical Examiner's Office, is offering fellowships in forensic and anatomic pathology. Training programs are individually adapted to background and experience.

For further information, write Joseph W. Spelman, M.D., Medical Examiner, 13th and Wood St., Philadelphia 7.

The Fourth International Goiter Conference.—The Fourth International Goiter Conference will be held July 5-9, 1960, in London, England, under the auspices of the London Thyroid Club and the American Goiter Association. The American Goiter Association plans to make available to worthy candidates a limited number of travel grants for participation in this meeting.

Application blanks are available from John C. McClintock, M.D., 149½ Washington Ave., Albany 10, N. Y., U. S. A.

Applications will be received until Jan. 1, 1960.

The Johns Hopkins University Research Position.—The Johns Hopkins University has a position open in a research laboratory concerned with the pathology of pregnancy and newborn infants. This would be suitable for a pediatrician, an obstetrician, or a pathologist. There will be an opportunity for doing research independently or in collaboration with a senior staff member. The person should also serve as liaison between research in the laboratory and in the clinical service. Those interested should write to Dr. Peter Gruenwald, Department of Obstetrics, The Johns Hopkins Hospital, Baltimore 5.

PERSONAL

Dr. Stuart A. Wallace Receives Award.—Dr. Stuart A. Wallace, Chairman, Department of Pathology, Baylor University College of Medicine, was named "Pathologist of the Year for the State of Texas" as part of the George T. Caldwell Award. The presentation was made on Jan. 24, in recognition of Dr. Wallace's long and distinguished service in the field of pathology.

Dr. Paul E. Steiner Goes to Institute for Cancer Research, Philadelphia.—Dr. Paul E. Steiner, of the staff of the Department of Pathology, The University of Chicago, has joined the staff of the Institute for Cancer Research, Philadelphia, as a senior member.

Dr. Charles E. Dunlap Reelected to Board of Directors of Oak Ridge Institute.—Dr. Charles E. Dunlap, Chairman of the Department of Pathology of Tulane University School of Medicine, has been reelected to a three-year term on the Board of Directors of the Oak Ridge Institute of Nuclear Studies at Oak Ridge, Tenn.

DEATHS

Sir Robert Muir Dies.—Sir Robert Muir, Professor of Pathology at Glasgow University for 37 years, died in Edinburgh, on March 31, 1959, at the age of 94.

Dr. Elise L'Esperance Dies.—Dr. Elise L'Esperance, long known for her contributions to the field of cancer, died on Jan. 21, 1959, at the age of 83.

Books

Symposium on Embryonic Nutrition (A Report from the Developmental Biology Conference Series, 1956). Dorothea Rudnick, Editor. Price, $3.25. Pp. 113. University of Chicago Press, 58th and Ellis Ave., Chicago 37, 1958.

This small book is a report of six papers and the discussion that followed their presentation, which was part of a conference on Developmental Biology, sponsored by the National Academy of Sciences—National Research Council. Participants were outstanding investigators in the field of embryonic nutrition from the United States and Europe; the subjects discussed included nutritional requirements and metabolism of the embryo, enzyme formation and activity, protein synthesis, and the use of antigens as tracers. Much of the material is concerned with the metabolic activity of the egg of the sea urchin and various fishes presented under the titles of Embryonic Energy Exchange, Metabolic Patterns in the Sea-Urchin Embryo, and Yolk Utilization in Fishes. Other papers included Antigens as Tracers of Embryonic Synthesis, Nutrient Necessities in Chick Development, and Enzymes: Formation and Growth. The material is concerned with the basic nature of these processes and tries to answer very fundamental questions related to cell metabolism. It should be of interest to all concerned with investigation of the intimate factors related to development and growth.

Environmental Influences on Prenatal Development (A Report from the Developmental Biology Conference Series, 1956). Beatrice Mintz, Editor. Price, $3. Pp. 112. University of Chicago Press, 58th and Ellis Ave., Chicago 37, 1958.

This small volume is a report of a session that was part of the Developmental Biology Conference series, which had the stated purpose of "contributing to clarification, order, harmonization and comprehensibility" of various subjects—in this instance the effect of environment on prenatal development. It was a symposium devoted to a discussion of the manner in which various teratologic agents affect the development of the embryo and fetus in utero, in which discussion some of the foremost investigators in the field participated. The experimental alteration of morphogenesis, the specificity of teratogenic agents and the multiplicity of their effects, the interaction of areas of growth in time and space, the nutritional requirements for morphogenesis, and the action of metabolic inhibitors were some of the subjects on which reports were presented. It is an excellent review of the material and should be of interest to everyone concerned with reproduction in any species.

The Birth of Normal Babies. By Lyon P. Strean, Ph.D., D.D.S., F.A.P.H.A. Price, $3.95. Pp. 194. Twayne Publishers, 31 Union Sq., New York 3, 1958.

This book does not warrant review in a scientific journal. It is amateurishly written by a dentist for prospective mothers, with the stated intention of banishing "fears, ignorance and superstition." It does just the opposite and intimates that any form of physical, physiological, or emotional "stress," with pregnancy itself interpreted as a "stress" state, can produce a malformed offspring. The book is full of the misstatements and half-truths so commonly found in the writings of people with an idea they wish to promulgate but without adequate factual knowledge to support their beliefs. The author's thesis is that any form of stress causes an overproduction of cortisone, which is teratogenic to the embryo in the early weeks of development. Evidence is accumulating that this may be true, but the philosophic discussions of the author and the many case histories he presents provide no additional support for this contention. The book will have no interest for the physician and in the reviewer's opinion is not to be recommended to prospective parents.

Histopathologie der Tumoren der Kiefer und der Mundhöhle. By Prof. Dr. E. Langer. Price, $11.80. Pp. 167, with 212 illustrations. Georg Thieme Verlag, Herdweg 63, (14) Stuttgart N (American Zone). (Agent in United States and Canada—Intercontinental Medical Book Corporation, Subsidiary of Grune & Stratton, Inc., 381 4th Ave., New York 16), 1958.

This is a concise and satisfactory outline of tumors of the jaw, salivary glands, and oral cavity. The book is primarily written for oral surgeons, dentists, and otolaryngologists. Emphasis is placed on black-and-white photomicrographs, and, indeed, they are well selected

and beautifully reproduced. The magnifications of all photomicrographs are stated. The text is restricted to the essential features of each individual tumor: age and sex incidence, location, morphological variations, and brief comments on the histogenesis. The author avoids the pros and cons of controversial questions and rarely presents his own opinion. As an orientation, the book is of help to the general pathologist. References refer to the older literature, mostly German.

Hæmatology. By D. A. G. Galton, Scientific Editor, and others. British Medical Bulletin, Vol. 15, No. 1, Jan., 1959. Price, $3.25. Pp. 880. Medical Department, The British Council, 65 Davies St., London W. 1, England, 1959.

This is a valuable collection of authoritative contributions by outstanding British hematologists. Subjects covered in the fourteen reviews include, among others, iron absorption, biosynthesis of hemoglobin, fetal hemoglobin, red-cell structure and metabolism in hemolytic anemia, acquired hemolytic anemias, treatment of the chronic leukemias, and chemistry of the abnormal human hemoglobins. As a whole, the reviews are accurate and up-to-date. They are not exhaustive, but do manage to include most pertinent contributions.

The Biology of Hair Growth. By William Montagna and Richard A. Ellis. Price, $15. Pp. 520. Academic Press Inc., 111 Fifth Ave., New York 3, 1958.

A symposium on the biology of hair growth was held at the Royal College of Surgeons in London in 1957, sponsored by the British Society for Research on Ageing, with the financial support of County Laboratories, Ltd., Stanmore, Middlesex. This book represents the papers that were read and discussed by laboratory scientists and dermatologists, together with some further chapters that make this a complete record of current knowledge of the subject.

Pathologists will be particularly interested in the sections on embryology, with new facts and interpretations by Hermann Pinkus; the anatomy of the hair follicle, with photographs of Van Scott's beautiful three-dimensional reconstructions; the chapters on electron microscopy by Mercer, Barnicot, and Birbeck; the growth cycle of the follicle by Bullough and Laurence, and the nature of hair pigment by Fitzpatrick, Brunet, and Kukita. Some pathologic conditions are discussed in this last chapter and in Van Scott's on the response of hair roots to chemical and physical influences; otherwise the papers are restricted to physiologic growth and differentiation.

The editor's concluding summary contains the excellent statement that epithelial cells phagocytose the dendrites of melanocytes, a very good description. This summary deserves the reader's confidence, but tyrosinase is erroneously printed for tyrosine in two sentences. The statement that pheomelanin could be formed by the oxidation of an *o*-aminophenol by dopaquinone, "produced by the oxidation action of tyrosinase or dopa" should read "action of tyrosinase on dopa."

The book is well balanced and complete; and, although the subject is discussed from many different aspects, there is no contradiction. The antithesis concerning hair neogenesis in normal skin and in special situations, including wound healing, is reconciled. What little repetition occurs is instructive. The interesting physiologic importance of the connective tissue papilla runs like a theme through the book.

This volume is, almost throughout, a work of scholarship that will need to be in the hands of the investigative pathologist and will be read with interest and profit by all histopathologists.

Color Atlas of Morphologic Hematology. Revised Edition. By Geneva A. Daland; edited by Thomas Hale Ham, illustrations by Etta Piotti. Price, $6.75. Pp. 72. Harvard University Press, Cambridge 38, Mass., 1958.

This is a well-written, succinct account of examination of the stained smear of peripheral blood. It is well indexed and has a well-chosen bibliography of 316 references. The morphology of peripheral blood cells is clearly presented, after which clinical information and detailed description of the 16 color plates are given. The color plates are beautifully drawn and reproduce faithfully the appearance of blood cells as seen in Wright's stained blood films. Unfortunately, as in most other color atlases, the plates are separated from their detailed description, which causes minor inconvenience to the reader in moving back and forth from the plates to the appropriate text section.

This book should be an excellent teaching aid for technologists, medical students, and residents.

The Effect of Advancing Age upon the Human Spinal Cord. By L. Raymond Morrison, M.D., with the collaboration of Stanley Cobb, M.D., and Walter Bauer, M.D. Price, $6. Pp. 127. Harvard University Press, Cambridge 38, Mass., 1959.

The delimitation of the range of the normal is ever a vexatious problem for those concerned with the interpretation of histopathological changes in the nervous system as they present themselves in run-of-the-mill human autopsy material. Truly adequate "normal" control material is difficult, indeed often impossible, to come by, and the assessments made by various pathologists in these matters are often highly impressionistic. This is likely to be the case especially where the observer is confronted with mild or chronic degenerative changes in the tissues of the nervous system.

It is the author's purpose in the present monograph, published posthumously, to provide a standard for changes in the tissues of the spinal cord which come with physiological aging. The observations are based upon the meticulous examination of 31 spinal cords from patients who presented no clinical evidence of spinal cord disease. Specimens from patients of the second through the ninth decades are represented in the series. Careful attention is given to all of the histological components of the spinal cord and the spinal roots: nerve cells, axis-cylinders, myelin, neuroglia, blood vessels, and meninges. The information recorded will be indispensable to all workers concerned with the finer details of spinal cord disease. The second section of the book, which is an atlas of the normal spinal cord in transverse section, segment by segment through its entire length, gives the book a much wider interest and appeal. It may be expected to supplement the standard "Atlas" of Bruce. The cell counts of the spinal gray matter and the subdivisions of these cells into multisegmental groups or columns are the best available.

The basic work behind the book had been completed by Dr. Morrison before his untimely death, but it was not possible for him to complete the preparation of the manuscript. Fortunately, this has been done by his colleagues, Profs. Stanley Cobb and Walter Bauer. The format of the book, which was published for the Commonwealth Fund by the Harvard University Press, is most pleasing, and the reproduction of the numerous illustrations is excellent.

Difficult Diagnosis: A Guide to the Interpretation of Obscure Illness. By H. J. Roberts, M.D. Price, $19. Pp. 913, with 99 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1958.

Roberts has been successful in his effort to orient "Difficult Diagnosis" on "an advanced postgraduate level for clinicians whose experience and skepticism will critically dictate to them its place in the analysis of difficult case material." Of its two sections, the first, and longest, is by far the most valuable. The author includes personal experiences, but relies heavily on recent medical literature. Well over 2,000 key references to articles of clinical importance make this an invaluable reference book. Excellent coverage is given to iatrogenic illness and obscure postoperative complications. Ninety-nine photographs, most of them obtained from standard reference books, are included; and, while admittedly limited in number, they constitute a fine collection of illustrations of dermatological problems or dermatological manifestations of systemic disease.

The second part of the book is a "Classification and Analysis of Useful Diagnostic Procedures." In it, various normal values are given for all standard and many unusual clinical procedures. Some notes of a technical nature about the various procedures are included, and "clinical clues" point out the significance of the results obtained, as well as many of the pitfalls responsible for false-positives and false-negatives. Some of these are so brief as to be of no value, but most of them are informative and interesting. Finally, the value of this book as a reference work is abetted by an "Index of Signs, Symptoms, and Laboratory Manifestations."

This book suffers from omissions or errors that might have been avoided had chapters been written by persons with interests in particular subspecialties. These are, however, limitations of any one person's ability to be an authority in all phases of medicine. It is surprising, in this era when standard references are each written by scores of authors, that one person would undertake such a monumental task as the writing of "A Guide to the Interpretation of Obscure Illness." It is remarkable to find that the result turned out so well.

Die Lungenarterienbahn bei angeborenen Herzfehlern. By Priv.-Doz. Dr. Karl Koehn and Dr. M. Richter. Price, $7. Pp. 112, with 52 illustrations. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N (American Zone). (Agent in United States and Canada—Intercontinental Medical Book Corporation, Subsidiary of Grune & Stratton, Inc., 381 4th Ave., New York 16), 1958.

The second publication of the series "Zwanglose Abhandlungen aus dem Gebiet der normalen und pathologischen Anatomie" is concerned with the changes in the pulmonary arterial bed in congenital heart disease. Forty-one lungs of patients with congenital heart disease were examined by means of serial sections. The histopathologic findings are described in detail and presented according to Bing's classification (congenital heart disease with increased and with decreased flow in pulmonary circulation and with equal flow in pulmonary and systemic circulations). In general, no specific changes related to any group were disclosed, though arteriovenous fistulae were commoner in the altered-pressure groups. The lesions of small arteries and arterioles are otherwise nonspecific.

The authors review critical past and current concepts of cause, mechanisms, and histopathology of secondary-pulmonary-artery sclerosis. The discussion of the causation is mainly devoted to hemodynamic, hypoxemic, infectious, allergic, and constitutional etiology. Neither morphology nor etiology is unique in pulmonary arteries of congenital cardiac disorders. The fascicle contains complete references. However, the authors' own investigations do not reveal significant new viewpoints.

The Atomic Age and Our Biological Future. By H. V. Brøndsted; translated by E. M. Huggard. Price, $2.75. Pp. 80. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1957.

This little book, apparently written for its propaganda value, has no scientific merit. H. V. Brøndsted is professor of zoology at the University of Copenhagen, working primarily in the fields of experimental embryology and tissue regeneration. In his work, he has become alarmed by some aspects of radiation and, though obviously not well versed in radiation, has chosen to give a series of alarmist lectures to the general public. This book is based on those lectures.

The book, written at a nonprofessional level, is a series of innuendoes, implications, and half-truths on the dire effects of radiation, all presented without scientific documentation. The author is on relatively safe ground in his early chapters, when he discusses basic aspects of radiation, early embryology, and mutations. But, in considering the "Visible Effects of Radiation on the Body," the author permits himself unproved and emotional statements, such as, in discussing the incidence of lung carcinoma at Joachimstal, "it is hardly to be doubted that radiation has its own large share in this tragedy," or "the advantage to be gained from luminous watch and clock hands was paid for by 41 human lives in a mere 20 years." In other areas he states that "small doses of a few r can gradually induce sterility" or "it is then established that the fetal mortality in the hypocenter [at Nagasaki] was appallingly high." In both these instances he has twisted known facts to his own purposes. And so the book goes through chapters on radiation genetics, drawing heavily on H. J. Müller's work, and finally concluding with a chapter on isotopes. The final thought in the book, while talking about radiostrontium, is that "the possibility cannot be excluded that by remote action mutations may also occur in the sex cells. Thus, from the point of view of future generations hydrogen bomb tests are unjustifiable."

It is unfortunate that Dr. Brøndsted has seen fit to publish his own emotions and prejudices. Certainly, he has neither the background nor the judgment to be writing in the field of radiation biology. Such publications only confuse and panic the public. Readers who are seriously interested in the public health problems of the atomic age are better advised to consult the recent "Report of the United Nations Scientific Committee on the Effects of Atomic Radiation," which represents the assembled thoughts of the world's recognized radiation biologists.

Haematological Technique. By E. M. Darmady, M.A., M.D., F.R.C.P., and S. G. T. Davenport. Price, $3.75. Pp. 244, with 27 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1958.

The first edition of this English hematologic laboratory manual was first published in 1954, when it was intended for use by hematology technicians. Several changes have been made to make this second edition possible. In the chapter on hemoglobinometry two pages about hemoglobin electrophoresis have been added, and the chapter on the investigation of coagulation

and bleeding defects has been expanded. A notable deficiency in the first edition was the lack of any systematic consideration of hospital serology. This has been corrected by adding four short chapters on this subject. The book has not been significantly enlarged, for the chapters on blood-bank organization and parasitology have been omitted. The great majority of the hematologic procedures performed in most laboratories are systematically considered in twenty-five chapters. Both the Ashby and the radioactive chromium techniques for determining red-cell survival are included. This was a notable deficiency in a recently published similar American manual. This book mentions briefly the theoretical backgrounds of most of the procedures considered and has an elementary discussion of hematologic disorders in the chapters concerned with the tests which are performed in the investigation of the diseases. This manual cannot be used as a "cookbook," because most of the tests are not described in sufficient detail. In many of the tests only the broad outlines of the performance of the procedure are given, and an inexperienced technician may encounter difficulty in performing some of the less common tests. This is the greatest defect in this manual. However, sprinkled throughout the book are "pearls" which this reviewer has not seen elsewhere—an example: the use of terpineol as a dehydrating agent after staining marrow with Romanowsky-type stains. This prevents the decolorization of the azure component which regularly occurs with alcohol or acetone. In spite of its limitations, many technicians may find this book useful. Its strongest points are its consideration of morphologic hematology and coagulation defects.

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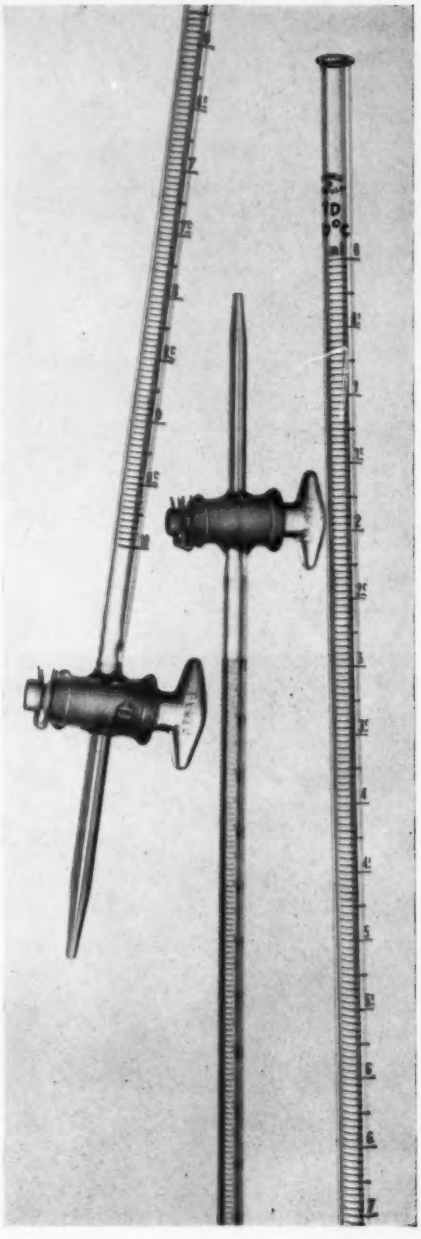

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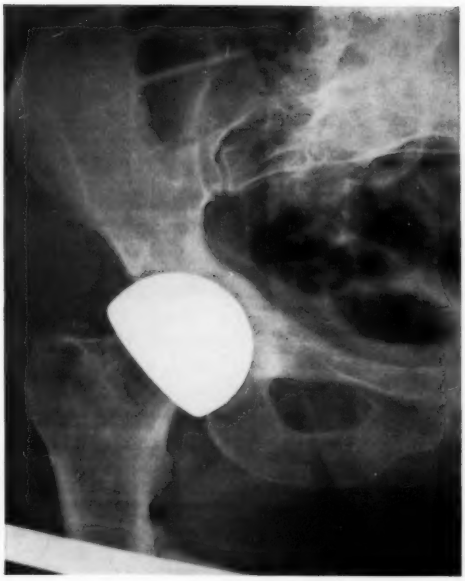

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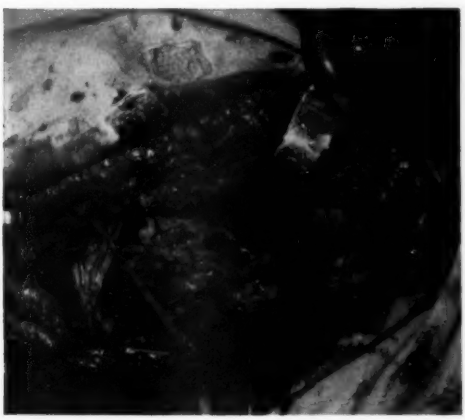

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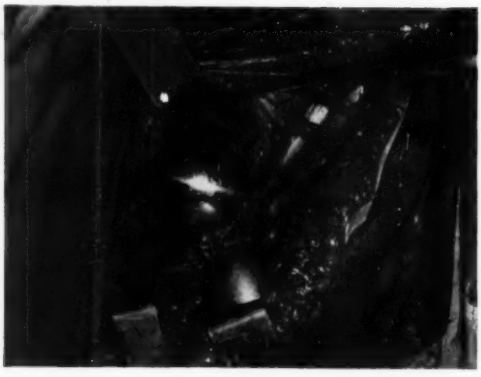

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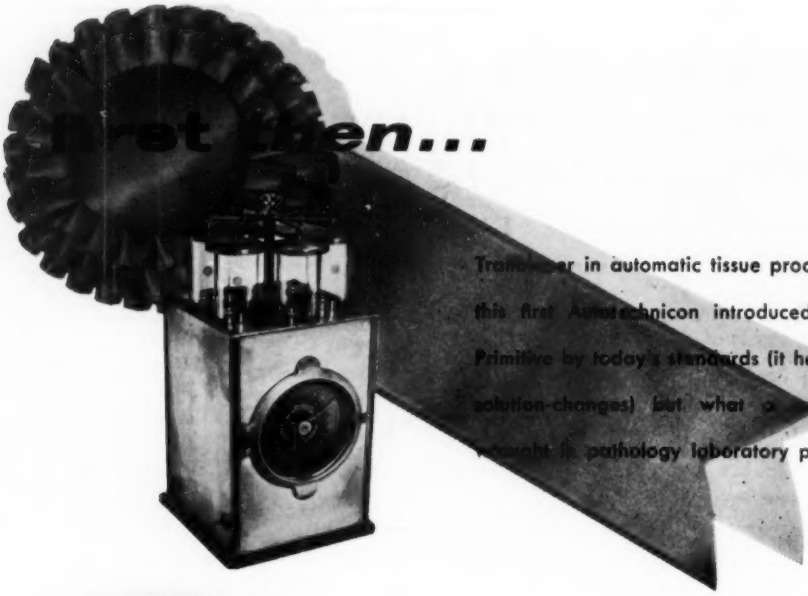

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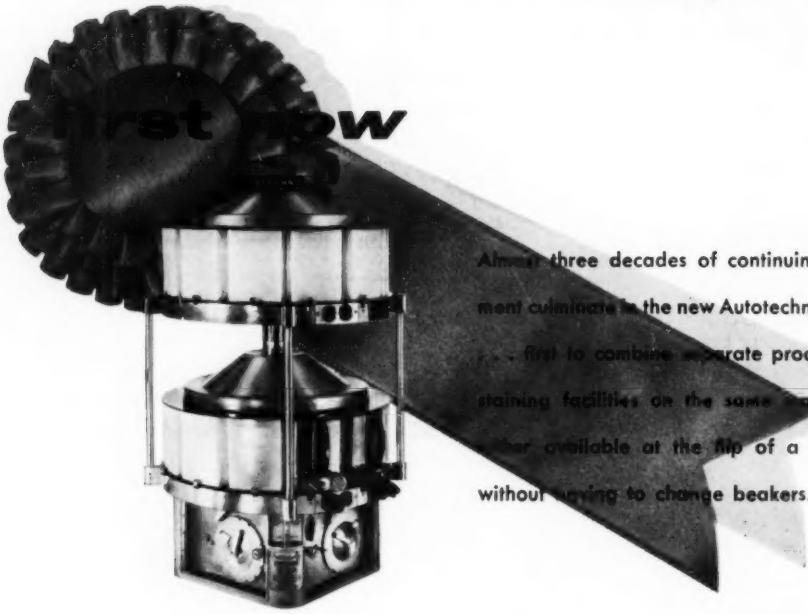

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